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DEGENERATIVE ARTHRITIS *

A COMPARISON OF THE PATHOLOGICAL CHANGES IN MAN AND EQUINES

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It is now known that many animal species suffer from joint diseases of both known and unknown etiology and that some of these are similar in character in all species studied. In a previous paper [1] we reported the results of an investigation which showed that a very prevalent condition among Army horses and mules in Panama, which had been considered a form of osteomalacia, and which caused a large amount of lameness, was in reality degenerative arthritis of undetermined etiology. It was also indicated that the condition in the horses and mules was very similar to the degenerative type of arthritis in man. We present in this paper results of a comparative study of the affection in man and the equine species.

Arthritis may be divided into two general types, inflammatory and degenerative. Both include diseases of known etiology. In the inflammatory group there are the specific infections, of which tuberculosis, gonorrheal and streptococcal arthritis are examples in man. In lower animals the arthritis of pyosepticemia or "joint-evil" due to *Shigella viscosa*, *Salmonella abortivo-equinus*, *Escherichia coli*, or streptococci, is the outstanding example of this type. In the degenerative group Charcot's arthropathy seen in syphilis [2]

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and also following traumatic nerve destruction [3] is the outstanding lesion of known etiology. In addition to these there are inflammatory arthritides sometimes resulting in ankylosis, of which the etiology is obscure though they are generally designated as "rheumatoid" or atrophic, and degenerative lesions of unknown etiology which, as they usually present clinical symptoms only in the later stages when compensatory bone production occurs, are then called hypertrophic arthritis.

This paper is based on 60 specimens from man and one or more joints from each of 54 horses and mules destroyed because of disabling lameness. Most of the specimens from man were from the knee and, with the exception of one or two from the surgical clinic, they were obtained at autopsy.

The various types of pathology of diseased joints in man have been described by Nichols and Richardson,[4] Allison and Ghormley,[5] Parker *et al.*,[6, 7] and others. The pathology is also presented in the papers of Keefer *et al.*,[8] and in the review prepared at the request of the American Committee for the Control of Rheumatism, by Hench *et al.*[9] Degenerative changes in the joints of horses and mules are described in the papers of Williams, Fisher and Udall,[10] Hare,[11] Bennet and Bauer,[12] Kintner and Holt [13] and others. Parker's papers describing the changes in the knee joint with advancing age [6] and in rheumatoid arthritis [7] serve to clarify the essential difference in the pathology of these conditions. The first paper described degenerative arthritis, the second the essentially inflammatory nature of rheumatoid arthritis. Although publications prior to Parker's described many of the changes of degenerative arthritis, none are as definite as his, and the others at times are confusing because of the inclusion of pathological features of other conditions.

Certain changes have been observed in the material on which this paper is based that were not described by Parker and concerning some of these we have found no references. These studies appear to indicate rather clearly the sequence of events in the joint changes so the lesions will be described in the order in which they apparently occur, or rather in the order of their severity, for some of them may not be progressive.

In order to define the pathological changes the normal histological structure of the joints in man and in horses and mules will

be briefly described. Starting at the joint surface there is, in young animals and children, a layer of cells whose nuclei are roughly parallel with the surface and which appears to be continuous with the surface layer of the synovial membrane at the margin of the joint. In adults this layer of cells cannot be made out on the bearing surface of the cartilage but in the same position there is a membrane-like condensation of protoplasm which limits the more or less homogeneous ground substance between it and the underlying cartilage matrix (Fig. 1). It appears, as has been stated by others, that there is no synovial membrane over the central bearing surface of the joint cartilage, yet when loss of substance occurs on apposed surfaces in degeneration of the cartilage, so that such surfaces are no longer in contact, a membrane may form over them which appears to be synovial in character. There is no traceable connection between these membranes and the peripheral synovia except where ulcers are near or at the joint margin (Fig. 2). Beneath the peripheral membrane of the normal joint surface the homogeneous cartilage begins and extends to the calcified matrix overlying the cortical bone. Near the periphery the cartilage cells have their long axes more or less parallel to the surface. As the distance from the periphery increases the axes assume more and more a vertical position with relation to the bone cortex, and especially in horses and mules, tend to be arranged in rows at right angles to the subchondral bone (Fig. 3). In man, even at ages prior to complete ossification of the epiphyses, the cells are arranged much less regularly and are more often found in groups of nuclei not all of which have independent capsules (Fig. 1). The cells, and especially the nuclei, are larger and apparently fewer in number near the calcified matrix in both man and equines.

In so far as we can determine from a few specimens, prior to the completion of epiphyseal ossification no calcium deposit is found about the cartilage cells in man or equines, as indicated by staining reactions in frozen sections of joint cartilage which has not been decalcified. However, as early as the 24th year in man, in this series, frozen sections stained by hematoxylin show zones of deep blue staining granules about the cell capsules. These are largest near the calcified matrix, decreasing to practical absence near the joint surface. These calcific zones have been found in

apparently normal cartilage at all older ages examined. Similar deposits were found in the normal cartilages of full-grown horses but not in those of a 10 months fetus. This finding suggests a change in the chemical composition of the cartilage about the time when ossification is complete.

The cartilage terminates abruptly at the calcified matrix. This is a layer of material having nuclei of the cartilage type and containing calcium which often appears in wavy striae parallel to the bone cortex, the stria joining the cartilage usually being the most densely stained with the blue of hematoxylin. Normally this layer is distinct at its junction with the cartilage, but projects into the bone cortex in irregularly shaped papillary-like masses which blend with the underlying cortical bone. Calcium appears to be retained in this matrix after decalcification processes have completely removed it from the normal bone beneath, in so far as staining reactions reveal its presence (Fig. 3).

Beneath the calcified matrix is the cortical bone, the character of which varies with the species, size and activity of the animal and differs in each bone and in different parts of the same bone. In general, large surfaces which bear the stress more or less evenly show little thickening or condensation of the trabeculae of the cortical bone. The central portions of the femoral condyles are examples. Localized areas where the subchondral trabeculae form compact bone with few marrow spaces occur at points of maximum stress, as in the grooves and on the apices of the ridges of the tibial tarsal bone and the tibia of horses and mules.

In judging as to the condition of the bone, therefore, these variations must be remembered, as must also the natural difference in bony structure of larger or smaller animals and also variations secondary to physical activity.

In fetal cartilage no definite line of demarcation can be made out between that portion which will remain cartilage and that which will be transformed into bone. Most of the blood vessels loop back at about normal cartilage depth from the periphery but occasional vessels approach nearer the joint surface. After the bone cortex is defined and the calcified matrix is laid no vessels are found in the cartilage, but in all types of cortex, from the least to the most dense, vascular tissue is found projecting from the marrow spaces into or even through the calcified matrix. These

projections are more numerous in cortical bone in which there is little peripheral condensation of the trabeculae (Fig. 3).

It appears to us that variations in density of the bone cortex have been misconstrued as changes due or secondary to degenerative arthritis, the lesions of which are prone to occur at just those points of stress where normally the bone is more dense. With these normal conditions and variations in mind the pathological lesions of degenerative arthritis, as observed by us in man and in horses and mules, will be described.

The mildest lesion seen consists of parallel grooves running in the direction of motion of the joint surfaces. These are relatively infrequent in horses and mules and rare in man. In the former they are found in the hinge joints like the tibiotarsal; in man on the patella, especially on the middle facets. In the equine species this may be the only type of lesion of a joint and it involves both joint surfaces, while in man other changes have always been present and the patellar cartilage alone may be affected.

Grossly the lesion consists of grooves of varying depth, the lateral margins appearing slightly raised. There is little change in color except in the deeper ones in equines in which the depth of the depression in some areas is dark red in color, the base being so thinned that the vascular underlying bone shows through. Hare,[11] in his study of this type of lesion, found that it consisted of ridges and grooves, the ridges of one of the apposed surfaces fitting into the grooves of the other. This we have been unable to demonstrate in our specimens. In fact, the depth of the groove often is made up of less dense tissue than the surrounding cartilage, indicating lack of pressure in this area. In man only shallow grooves have been seen (Figs. 4 and 5).

Microscopically a section across the long axis of a groove shows a depression of the surface of the cartilage with relatively slight bulging or elevation of the lateral margins. The membranous surface layer may be swollen in the depth of the groove. The cartilage cells are somewhat disturbed in arrangement and more separated in the matrix beneath the marginal bulging. The cartilage beneath shows more or less fibrillation, the long axes of the fibers being at right angles to the joint surface, the fibrillated material extending a varying distance toward the cortical bone (Figs. 6 and 7). Deeper grooves show correspondingly greater changes

both in the character and in the degree of the cell arrangement. Groups of nuclei in a single capsular space are frequent and fibrillation is more pronounced. Occasionally splits or clefts are formed along the fibrils, extending varying distances into the cartilage (Fig. 8). These may reach the cortical bone and sometimes penetrate into it along the vascular tissue projections which extend from the bone into or through the calcified matrix. When this occurs there is a necrosis of the subchondral plate and a production of fibrous tissue in the spaces between the bone trabeculae. This may go on to the formation of cartilage and osteoid tissue, while lateral to such clefts bone production, beginning as an increase in thickness of the calcified matrix, may take place and extend into the cartilage. In this manner a bony thickening of the cortex may occur.

These clefts and accompanying or subsequent changes are much more frequent following other types of degenerative change described below. In our material lesions were found in the subchondral bone only when cartilage changes had reached the bony cortex. We are therefore unable to confirm Hare's hypothesis that focal necrosis in the bone is the primary change. In our specimens the bone changes have been obviously secondary to those of the cartilage.

"Blister" formation is somewhat more common than grooving and may be seen in any joint. Equine joints have furnished the best examples. The first changes noted grossly are small smooth elevations of the surface of the cartilage without color change. These become somewhat flattened at the top, the margins more sharply defined, and the color paler than the surrounding tissue. Rupture usually occurs and the peripheral layers become shreds or fringes projecting above the surface. It appears that coalescence of these blisters forms fringe areas of varying size (Figs. 15 and 16).

Microscopically the early stages in formation are best studied in frozen sections of the joint cartilage. Serial sections of an early elevation show in the center an irregular space bounded by fibrillated cartilage surrounded by a zone, more or less fibrillar, with cells more widely separated than normal (Fig. 12). This change gradually decreases to blend finally with the normal cartilage beyond the swollen areas. Study of a number of these early lesions,

which are located in the peripheral third or half of the cartilage, suggests that the primary change is a focal edema, followed by fibrillation and "blister" formation (Fig. 11). Rupture of such blisters gives rise to fringes. These consist of strands of fibrillated cartilage projecting above the surface, the longer ones being attached at the periphery of the blister, the shorter extending from the base of what now may be termed a small "ulcer" or erosion.

Occasionally on the same joint surface on which the discrete blister formations are seen, shallow pits occur which have slightly raised edges and a smooth surface only slightly paler than the unchanged cartilage. Microscopically these are covered by partly hyalinized fibrocartilage continuous with the upper layers of the normal cartilage (Fig. 13). Beneath the surface layers, and extending a varying distance toward the cortical bone, the cartilage is more or less fibrillated and the nuclear arrangement is disturbed. Such pits appear to result from blister formation followed by loss of the fluid but without fringes being formed, as is the case in those that have ruptured.

Considerable areas of this "fringed" cartilage are found which, as stated above, may have resulted from the coalescence or extension of the blister areas, but may also have originated otherwise, though other mechanisms or sequences of events have not been evident in our material. Traumatism of the apposed surface by the frayed tissue may well account for the fringed area on that side without the preliminary blister formation. Figure 14 shows changes in the cartilage of the proximal end of the humerus of a mule apposed to an eroded, fringed glenoid fossa. The small pale areas are fibrillated, almost myxomatous tissue. In some areas there were some membranous fringes and the entire cartilage showed fibrillation and abnormal arrangement of the cartilage cells.

Clefts extending toward or into the cortical bone occur, as in the case of the grooves. These are often close together along the surface and extend but a short distance into the cartilage, while others, more widely separated, extend deeper, forming irregular masses of cartilage attached at the base to the calcified matrix. Beneath, and in the fringes, the alignment of the cells is markedly disturbed and cell nests are often abundant. Practically always both apposed surfaces show changes though these are usually dif-

ferent in extent, sometimes in type, or both. Grossly the uniformly fringed areas are described as erosions. Relatively low magnification (× 10), however, shows the essentially villous character of the bases which is confirmed by microscopic sections.

The further progress of the erosive degenerative process is characterized by a necrosis and loss of substance of the surface of the ulcer so that the remaining tissue is thinner than the surrounding cartilage. As this loss of substance continues the lesions appear as irregular, discrete and confluent areas eroded to varying depths below the surface of the normal cartilage (Fig. 19). The bases lose the villous character and become dingy gray in color. Such erosions or shallow ulcers were by far the most frequent type of lesion found by us in equines (Fig. 18). They were present in some joint of practically every animal autopsied for this investigation, all of which were destroyed because of disabling lameness. In man the fringed type was the most frequent (Fig. 15). In some of these the fringes projected above the surface but in many there was loss of substance so the fringed bases were definitely below the normal cartilage level. Most of the human bones, however, came from individuals who had given no history of joint disease.

The bases of the deeper ulcers, as observed in horses and mules, have been covered by a layer of dense fibrous tissue though masses of fibrillated hyaline cartilage may remain projecting from the underlying bone. The bone itself has never been bared. In man several advanced cases have shown areas of bare bone though in each instance portions of the surface were covered by fibrous tissue and fibrous and fibrillated hyaline cartilage (Fig. 17). When bone is bared it may be eburnated, but also may show thinning of the cortical layer and actual absence of bony cortex and calcified matrix. The bases of such areas consist of ends and small bridges of trabeculae between masses of fibrous and hyaline cartilage. In such cases vascular fibrous and osteoid tissue extends varying distances into the cortex, replacing the fat marrow between trabeculae (Fig. 20).

Frozen sections of undecalcified cartilage of affected joints show considerable variation in the amount of visible calcium as indicated by staining reactions. The cells near the calcified matrix showed the most but the distribution was not uniform. In some

areas in and near erosions and fringed areas none of the granular deposit may be seen. Further, in apparently normal cartilage, especially in the later age groups, the distribution was often uneven.

The ulceration and loss of substance usually involves both apposed surfaces which then cease to be in contact. Such areas no longer carry their proportion of the stress of the joint, therefore the remaining cartilage must carry the entire load. There is malocclusion of the joint. In such joints hypertrophic changes occur which appear to be compensatory in character.

The first change appears to be hypertrophy, or swelling of moderate degree, of the remaining cartilage at the margins of the bearing surface and extension of the cartilage over the edge of the joint surface, especially in such joints as the patella-femoral. The new or extended cartilage is fibrillated and the cell arrangement is distorted. Then bone formation starts from the calcified matrix and extends into the cartilage in the same manner as occurs beneath ulcerated areas on the original joint surfaces (Fig. 8). As the bone production continues vessels from the marrow enter the process and eventually the new bone becomes continuous with the old and appears of similar or like structure (Figs. 21–24). It has been suggested (Parker [6]) that the pressure in the maloccluded joint forces the bone outward to form these lips or shelves. Except that the pressure must have an influence in the direction the new bone formation will take in relation to the old bone, it does not appear from our material that this is so. That it is compensatory is indicated by the occurrence of the same process directed against the pressure of the joint as seen in Figures 23 and 24, where it seems that the bone is produced in an effort to compensate for the loss of cartilage substance.

Masses of these shelves composed of cartilage, with or without some bone formation, may fail to be firmly united to the bone. In such instances they become pedunculated and later may be separated and free in the joint as "joint mice" (Fig. 21). Nutrition of these free masses appears to be fairly well maintained though in the larger ones the central ossified portions may become necrotic. This maintenance of structure indicates that the nutrition of joint cartilage, to a great extent at least, is from the synovial fluid.

Hypertrophic lesions were neither numerous nor severe in the

horses and mules of our series. These animals are destroyed before the occurrence of such advanced changes as are found in man. Masses of cartilage in the capsule and at the joint margin, however, were occasionally found.

The semilunar cartilages of the knee show degenerative changes more or less in proportion to the degree of change in the cartilage of the apposed femur. These menisci are formed of bundles of fibers embedded in a hyaline matrix, the long axis of the fibers being roughly parallel to the contour of the meniscus. The cross section is triangular, the apex toward the center of the joint area, the base at the periphery. The base is attached by fibrous tissue to the margin of the tibial condyles and the fibrillated ends are attached to the central portion of the bone between the condyles. The medial meniscus is longer and smaller in cross section than the lateral one. The changes in degenerative arthritis are: first, fringes extending onto the central portions from the apices of the menisci; then fibrillation or rather loss of the hyaline matrix so that the cartilages appear as masses of fibers, which may rupture so that the cartilage appears fractured; and finally considerable portions may disappear (Figs. 25 and 26). These changes are usually more advanced in the larger medial meniscus and occasionally in man may be advanced with only relatively minor degeneration of the cartilages of the femur. As observed in our material, they were seldom of advanced degree in equines. In joints in which the menisci show advanced degeneration with loss of substance, new cartilage and bone formation may occur from the intercondylar eminence at or near the attachment of the lateral meniscus. Such formation would appear to be compensatory in that it would tend to limit the lateral motion of the femur on the tibia which the degenerated menisci no longer serve to prevent.

Changes in the synovial membrane lining the joint cavities have not been marked except in a few of the advanced hypertrophic cases in man. Vascular papillary projections from the synovial membrane are practically constant. These are of small size in most mildly affected joints, longer and more abundant in those showing hypertrophic changes. Occasionally in both man and equines there are relatively large (1–2 cm. diameter) pedunculated cystic masses covered by synovial membrane. Occasionally,

attached to and apparently arising from the capsule at its attachment to the bone, pedunculated and sessile masses of cartilage are found evidently arising from this membrane. Rarely such a cartilaginous mass is embedded beneath the synovial membrane in the inner tissues of the capsule.

In advanced hypertrophic cases the synovial fluid may be dark in color and gelatinous in consistence. When this condition has been found in equines there has usually been some evidence of recent injury complicating the disease process.

Discussion

Grooves and blisters, especially the latter, appear from our experience to be the primary and earliest lesions in degenerative arthritis. Grooves are best seen in equines. This type of lesion is easily overlooked in human joints. Practically all writers have described the fissures, clefts and fringe formation, and the extension of the clefts into the subchondral bone. The relation of these to blood vessels has been noted. We have not seen evidence of fracture of the bony plate as noted by Parker but fractures might well be caused by "joint mice" caught between the joint surfaces. As a result of the study of serial sections, these openings from cartilage to marrow appear to be the result primarily of a cartilage fissure extending through the calcified matrix along vessels which appear normally to extend to the cartilage from the marrow beneath. The clefts are localized; that is, the extension into the bone rarely exceeds 1 mm. in the plane at right angles to that of the section. Eight or ten sections at 25 μ often show both termini of these lesions.

Pressure may have something to do with the process in the early stage but the condition may continue after pressure has been removed by loss of substance from both apposed surfaces. Moreover, when new bone is formed above the previous normal level of the bony plate evidence may remain beneath the new bone cortex in the form of fibrous, cartilaginous or osteoid tissue which has not been replaced by fatty marrow. It appears that when the process is complete, as shown in Figures 23 and 24, a normal marrow eventuates as in primary bone formation. We are simply viewing various stages in the breakdown and repair of the joint structure. Therefore, it appears reasonable that abnormal tissues, remnants

of the repair, should be found beneath the joint surface after the process in the immediate area reaches a stage which, though imperfect as compared to the normal, is practically complete or stationary. It is necessary to view more than one plane in studying a three dimensional process if erroneous conclusions are to be avoided.

Several writers have mentioned the increased density of the bone beneath the degenerated areas, usually referring to it as compression of the trabeculae. Some also speak of rarefaction or osteoporosis as a feature of the process in the subchondral bone. As mentioned above, there is marked variation in the character of the subchondral bone. We have been unable to demonstrate in the subchondral bone either increases in density or porosity which could be considered a part of the disease. Certainly these are not early changes. Neither has vascular change nor the necrosis in the bone as described by Hare been evident in our material prior to advanced degeneration of the overlying cartilage.

Increase in density of subchondral bone occurs in areas where the physical nature of the joint indicates that the greater stresses occur. It is possible that due to this density the nutrition of the lower portion of the articular cartilage, which is believed to receive its nutrition from the underlying bone, receives less as a result of such density and relative avascularity and thus is rendered more susceptible to degenerative changes. However, the peripheral portions of the cartilage receive nutrition from the synovial fluid, as is indicated by the condition of the cartilage in "joint mice." From our material it appears that the primary degenerative change starts in the peripheral half or third of the cartilage and this is supported by the studies of others. It thus seems more logical to assume a change in the character of the synovial fluid, rather than in the subchondral bone, as responsible for the primary degenerative process.

Eburnation of the bared bony cortex occurs in advanced lesions in man but such eburnation appears to be the result of new compensatory bone formation which commences in the calcified matrix and extends into the cartilage. Such bone, kept bare by the frictional trauma of an apposed bare bone, may show a dense cortex, often interrupted by areas of imperfect bony closure of the bony plate as seen in Figure 20.

The productive (hypertrophic) changes are similar in man and equines but have been seen in advanced stages only in man. Their compensatory character has been indicated. There has been some argument as to the origin of the new formation of bone and cartilage but in the specimens available to us there seems to be but one source and that is the calcified matrix and subchondral bone. Even in advanced lesions we have not seen evidences of periosteal bone formation or evidence that the connective tissues at the margin of the joint enter into the productive process. Even at the margins where the lips or shelves are formed there is little evidence of new cartilage formation. Instead, there is evidence of proliferation of the cartilage cells near the calcified matrix while the peripheral hyaline cartilage appears edematous or swollen rather than proliferating. In some instances fibrous tissue from the region of the joint margin and capsular attachment extends over a lip or shelf of bone. This fibrous tissue seems to be proliferating and there is sometimes a suggestion of the formation of fibrocartilage but such pictures are unusual in degenerative arthritis and lead to a suspicion that some other factor is present. This construction is the common one in inflammatory arthritis, including the so-called rheumatoid variety in which the fibrous tissue mass extends as a pannus onto the surface of the joint cartilage; the cartilage degenerates, eventually may disappear and fibrous and finally bony ankylosis eventuate.

We have not examined phalangeal joints in which Nichols and Richardson described periosteal bone formation. In these joints the periarticular cartilage masses known as Heberden's nodes are large, as compared to the joint, and it would be difficult to decide their origin because their peripheries blend with the periosteal structures along the shafts of the bones. Certainly in the weight-bearing joints of our series, of all grades of severity, especially in the human specimens, we have not been able to determine periosteal bone formation. In equines this occurs in spavin, for instance, which is primarily not a disease of the joint cartilages, but there is bone production by the periosteum in the region of the capsular attachment which may extend over the joint area and interfere with motion. Spavin may be concurrent with but does not appear to be a part of degenerative arthritis.

The number of individuals, human and equine, that have been

examined routinely at autopsy for joint disease is still too small to determine the incidence of degenerative changes in the joints. Fifty-four Army horses and mules over 10 years old, destroyed because of lameness, had many lesions each in various joints but the extent and severity of the lesions did not always correspond with the clinical findings. That is, there were instances in which the most advanced or extensive lesions were in joints that had not been considered affected, and minor lesions in many joints other than those responsible for the symptoms. No lesions were found in a 6 year old pony that died of encephalitis.

These findings in equines are in accord with those in man. Only 5 of our 7 advanced cases with lipping and other hypertrophic changes had complained of their joints. Two of 18 cases with rather extensive erosions but no definite hypertrophic change had vague symptoms. Forty of the 48 persons whose knee joints were found affected did not complain of joint trouble. Twenty-two of the 40 had mild lesions, 16 had moderately extensive erosions and 2 had advanced lesions with hypertrophic changes on both patella and femur.

The human series of Keefer, Parker, *et al.*,[8] consisted of knee joints from 100 persons, dead of various diseases. In the combined groups (Keefer's and ours), no lesions were found in 7 in the first two decades of life. Lesions were present in 1 of 2 in the third decade, 5 of 7 in the fourth, 15 of 16 in the fifth, 36 of 37 in the sixth, all of 42 in the seventh, 26 of 27 in the eighth and 15 of 16 in the ninth decade. The findings in even this small number indicate that lesions of degenerative arthritis may occur at any time after the bone and joint structures are completely formed and that after the fifth decade is reached few persons are free from these changes. It will require the examination of a number of different joints of several thousand persons in general autopsy services to determine the incidence of this disease, especially because so few of those affected have clinical symptoms. Roentgen examinations are of great value in determining the presence of relatively early lesions in such joints as those of the vertebrae and fingers. They show ulcers on the condyles of the femur when they are deep enough and can be X-rayed in profile but do not show lesions of the patella and patellar fossae until the hypertrophic changes of the advanced process are present.

Clinical symptoms are of some value. In the inflammatory conditions, including the so-called rheumatoid arthritis, pain, tenderness and some fever are present early and often usher in the process, while in pure degenerative arthritis the process is usually advanced and secondary hypertrophic changes have occurred before symptoms are sufficiently severe to send the patient to a physician.

The seven advanced lesions in our series were well distributed in the age groups. The youngest was 48, the oldest 87. Both of these and two in the eighth decade, age 73 and 76, had syphilis. All seven had arterial disease and three had gangrene of toes. In our subclinical group as a whole, although the numbers are small, the extent of the area involved appeared to increase with advancing age, but exceptions were present. Work done so far suggests that starting at about age 20, the incidence rises with increasing rapidity for the next 20 years. Thereafter the changes are found in practically all. Increase in the extent of the areas involved appears to increase with age but increase in severity — or perhaps better stated as rapidity of progression of the lesions — seems to be due to other factors than age. One is reminded of the great variations in the extent and rapidity of progress of the arterial degeneration in atheromatous arteriosclerosis.

There is little evidence as to the cause or causes of the primary degenerative change. The age incidence suggests that metabolic or rather chemical or physico-chemical changes in the cartilage render it more susceptible to damage by normal wear and tear. The early stages of blister formation bear out this hypothesis. The subchondral bone cortex and the calcified matrix are formed at the limit of the vessel loops from the bone. The formation of this limiting line is gradual. In early childhood the end of the bone is largely cartilaginous and no obvious dividing line can be made out between that portion of the cartilage which will remain as such and that which will eventually become bone. The completion of the bone cortex and the superimposed calcified matrix may well mark a quantitative change in the nutrition brought to the joint cartilage. There are some data on this point, though how such chemical and chemico-physical changes as have been found are related to degeneration of the cartilage is unknown.

Hoffmann, Lehmann and Wertheimer are quoted by Bywaters [14]

as finding that the glycogen content of cartilage varies with the kind of cartilage and the age of the animal, also in different individuals, variations being between 0.02 and 0.6 per cent. It was also indicated that there is a diminution with age and with increasing calcification of the cartilages.

The calcium content of cartilage can scarcely be determined with sufficient accuracy for comparative studies because of the impossibility of obtaining uniform specimens for analysis. Unless samples include the base as well as the periphery of the cartilage without the inclusion of underlying calcified matrix and bone, and this is practically impossible, comparative analyses would be of little value. Our studies of frozen sections indicate that there is a considerable variation in the amount of visible calcium between the deep and superficial portions of the cartilage and between the cartilage of children and adults. Further, there is much variation in the evenness of its distribution in the cartilages of the later age groups. Whether this variation is quantitative chemically or whether it is one of the physical-chemical state of the calcium, we do not know. The degenerative changes, especially the splitting, suggest a loss of elasticity. Perhaps analogous conditions are the increase in calcium found accompanying loss of elasticity in the aorta and lens of the eye. Disturbance of chemical equilibrium might cause a change of the calcium from a colloid to an insoluble state, while simple dehydration, causing a relative increase in the calcium content, might be responsible for lack of cartilage elasticity. Certainly degenerated cartilages appear dry as compared with the normal, but comparative analyses for water content would be as difficult as those for calcium. Further, the cause of dehydration, if existent, might well be related to changes in osmotic pressure caused by decrease in salt content or change in the chemical-physical state.

Believing that a dietary deficiency with mineral imbalance was the cause of the lameness in the group from which our equine cases were taken, their diet was modified by adding alfalfa hay, which corrected the calcium deficiency, balanced the $CaO–P_2O_5$ ratio and added vitamins, especially vitamins A and D. A number of our cases had been on this improved diet for 2 years but we were unable to make out any evidence of a repair process other than the compensatory hypertrophic changes described. The period was

not sufficiently long to determine the effect of the new diet on the incidence of clinical lameness.

Conclusions

1. Degenerative arthritis is a definite disease entity of unknown etiology which commences as a degeneration of joint cartilage and involves bone only secondarily.

2. It exists in many animal species and is an important cause of disability in man and equines.

3. The lesions in man and horses and mules are practically identical in character, though the most advanced lesions are not ordinarily found in equines because the resulting disability causes them to be destroyed before the changes reach such a stage.

4. Though a relatively few examinations have been made, the lesions of degenerative arthritis have not been seen in man prior to the third decade, but after this their incidence increases rapidly so that they are almost universal when the fifth decade is reached.

5. A relatively small proportion of the lesions give rise to symptoms but this proportion increases with advancing age as does the extent of the areas involved.

6. Involvement of the cartilage alone often appears to be symptomless. When pain occurs it probably usually results from pressure on subchondral bone which has either been denuded of cartilage or more or less damaged by extension of the degenerative change. In those cases in which loose pieces of cartilage or bone (joint mice) are present in the joint, pain may be produced as a result of their getting between the joint surfaces.

7. Loss of cartilage substance, causing malocclusion of the joint, is followed by bone production from the calcified matrix and subchondral bone. This appears to be a compensatory effort to replace the degenerated cartilage with bone and, though secondary, has given the name hypertrophic arthritis to the disease. This new bone formation is rare in man except in the clinical disease in which it is usually present.

8. Symptomatic degenerative arthritis occurs in equines from less advanced lesions than in man. It appears that the greater the physical activity of the individual, man or animal, the more serious does this condition become.

9. While the area of cartilage, the number of joints involved,

and the incidence of clinical arthritis increase with advancing age, rapid progress of the disease to severe disability appears to be due to other factors as it may occur in the fifth decade or earlier, as well as in later life.

10. Ankylosis, fibrous or bony, does not occur in this condition though compensatory bone formation may be responsible for limitation of joint motion.

NOTE: The authors are indebted to Lieut. Col. H. W. Mahon, Capts. Karl R. Lundeberg and Dwight W. Kuhns, Medical Corps, for specimens from man, and to Lieut. Cols. C. W. Greenlee, C. C. Whitney and D. H. Mallan, V.C., for specimens from equines, and to Sgt. C. M. Margeson and Privates 1st Class John Austin and Elmer West for technical assistance.

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DESCRIPTION OF PLATES

PLATE 48

FIG. 1. Normal human joint cartilage from condyle of femur. Note irregular distribution of cartilage cells.

FIG. 2. Ulcer on femoral condyle. Base covered by synovial membrane which shows cyst formation. Calcified matrix thin with bony spur extending into cartilage to right of center.

FIG. 3. Normal equine lower cartilage, calcified matrix and cortex of subchondral bone. Note vessels penetrating nearly through calcified matrix which shows striae of calcification (*c.f.* Fig. 1 in which calcified matrix is not striated). Both types are found in man and equines. Note regular arrangement of the cartilage cells.

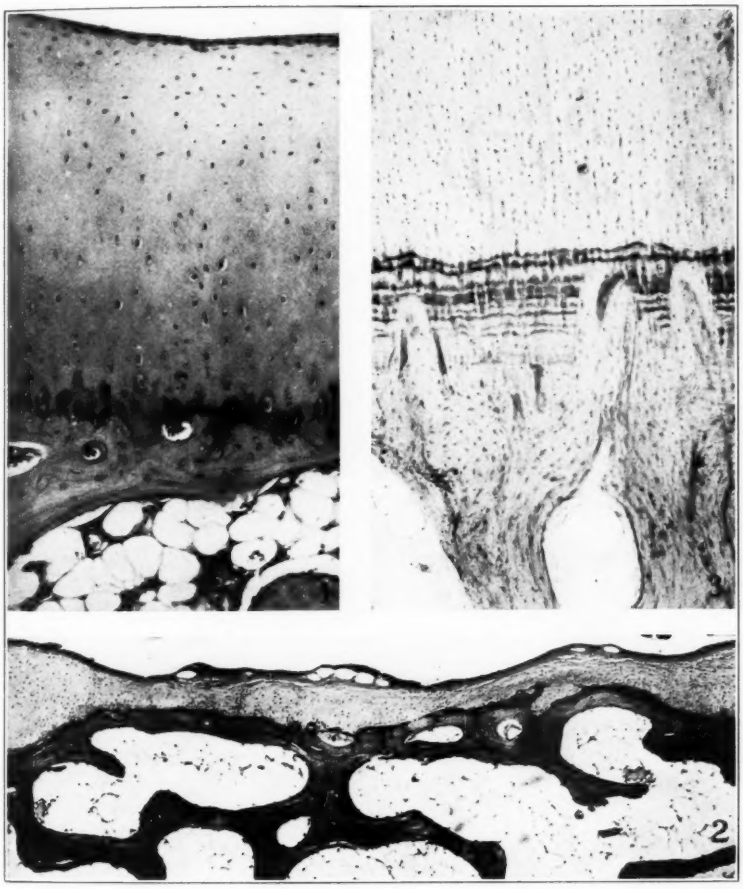

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PLATE 49

FIG. 4. Shallow grooves and "blister" in human patella.

FIG. 5. Shallow and deep grooves in the cartilage of the distal end of the large metacarpal bone of a horse. The darkest streaks showed deep red at the base.

FIG. 6. Shallow grooves in human joint cartilage cut across their long axis. Note soft tissue in base of the one at the left.

FIG. 7. Shallow groove in equine joint cartilage.

FIG. 8. Deep groove in equine joint cartilage. A cleft extends from base of groove into cortex of bone. Vascular fibrous tissue replaces bone about the cleft and extends into marrow spaces at lower right. Beginning bone formation replaces calcified matrix and extends into cartilage. Nuclear arrangement of cartilage is disturbed and there is necrosis of the fringed surface on the right margin of the groove.

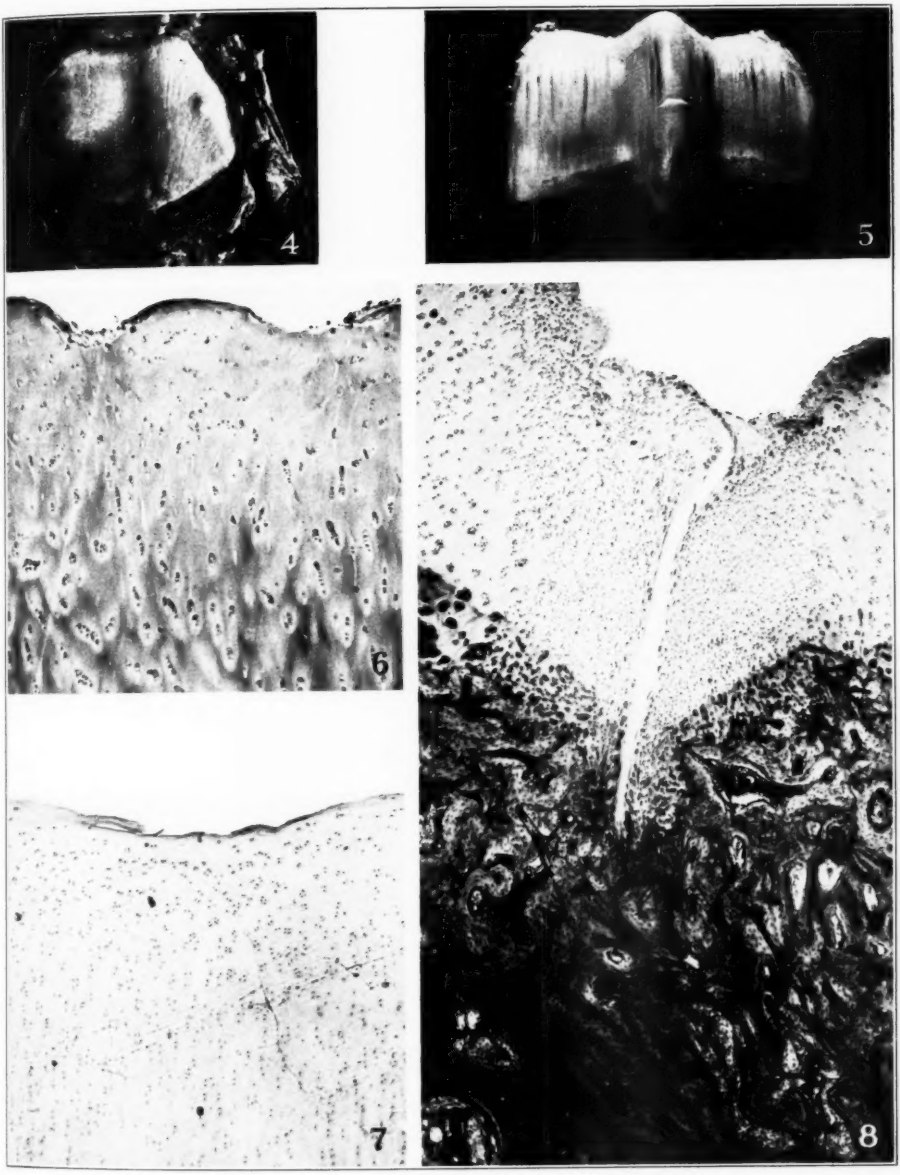

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PLATE 50

FIG. 9. Large "blister" on a human patella.

FIG. 10. "Blisters" and "pits" in cartilage of an equine patella.

FIG. 11. Mound of edematous cartilage on left and ruptured "blister" on right. Frozen section from equine patella cartilage which grossly was similar to specimen shown in Fig. 10.

FIG. 12. Early "blister" formation. Frozen section of equine patella cartilage.

FIG. 13. "Pit" in equine patella cartilage from specimen similar to that seen in Fig. 10.

FIG. 14. Membranous fringes on the surface and multiple areas of pale, almost myxomatous tissue in cartilage of equine humerus which was apposed to a fringed glenoid fossa.

FIG. 15. Fringed eroded patella fossa, upper left, and deep ulcer on condyle, lower left, of human femur. Patient had not complained of joint symptoms.

FIG. 16. Fringe areas and a few small "blisters" on femoral condyles of a mule. Animal had been affected with shifting lameness and many joints showed degenerative arthritis.

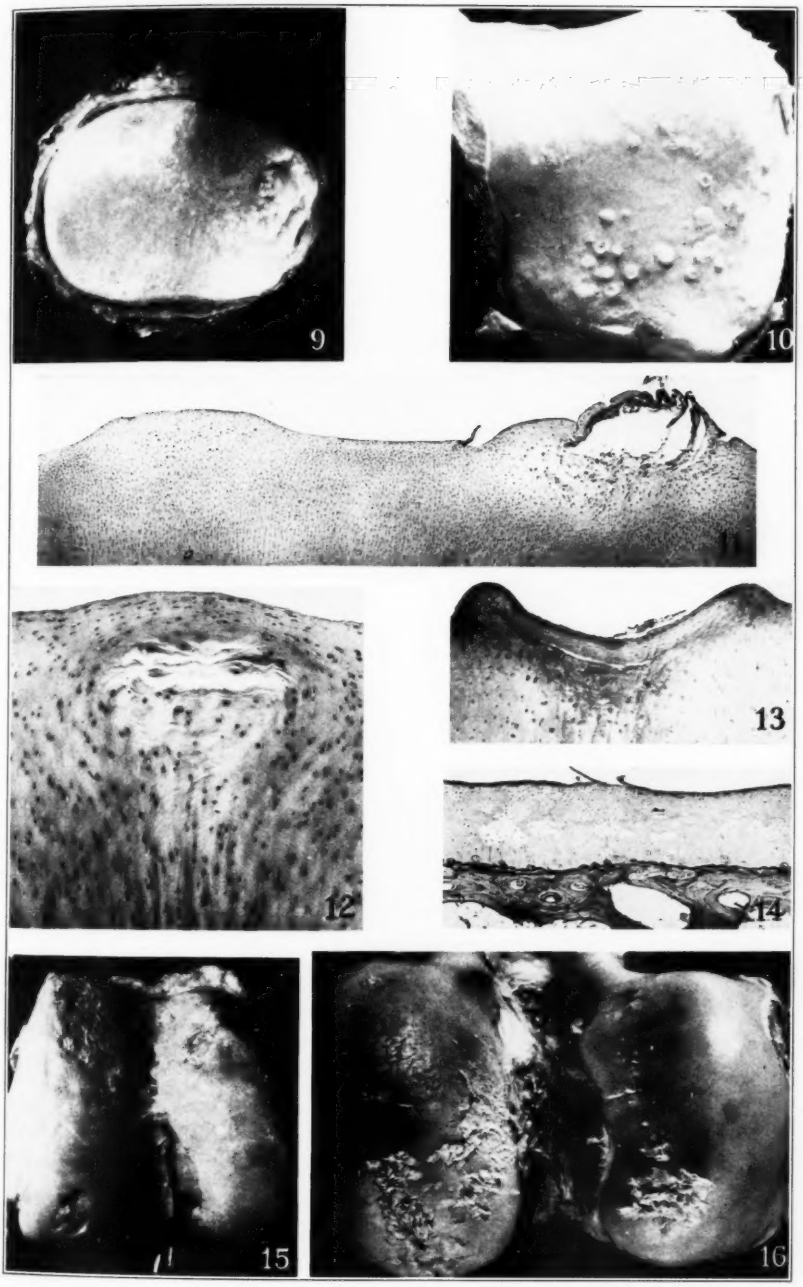

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PLATE 51

FIG. 17. Advanced degenerative arthritis with hypertrophic changes (see Fig. 21 for lateral view). Lower end of human femur. Condyles are partly denuded but there is little eburnation. Note island of swollen cartilage in the ulcerated area on the right.

FIG. 18. Erosions in the cartilage of the patella fossa and femoral condyles of a mule destroyed for lameness.

FIG. 19. Ulcer in human cartilage showing marked fibrillation fissures or clefts and bone formation from region of the calcified matrix. Note that there is no change in the density of the subchondral bone.

FIG. 20. Section from human joint which showed ulceration similar to that seen in Fig. 17. Ulcer surface composed of ends of trabeculae and masses of fibrillated cartilage. Marrow spaces near surface are filled with fibrous and osteoid tissue. There is lipping at the margin from new bone growth which is covered with swollen fibrillated cartilage.

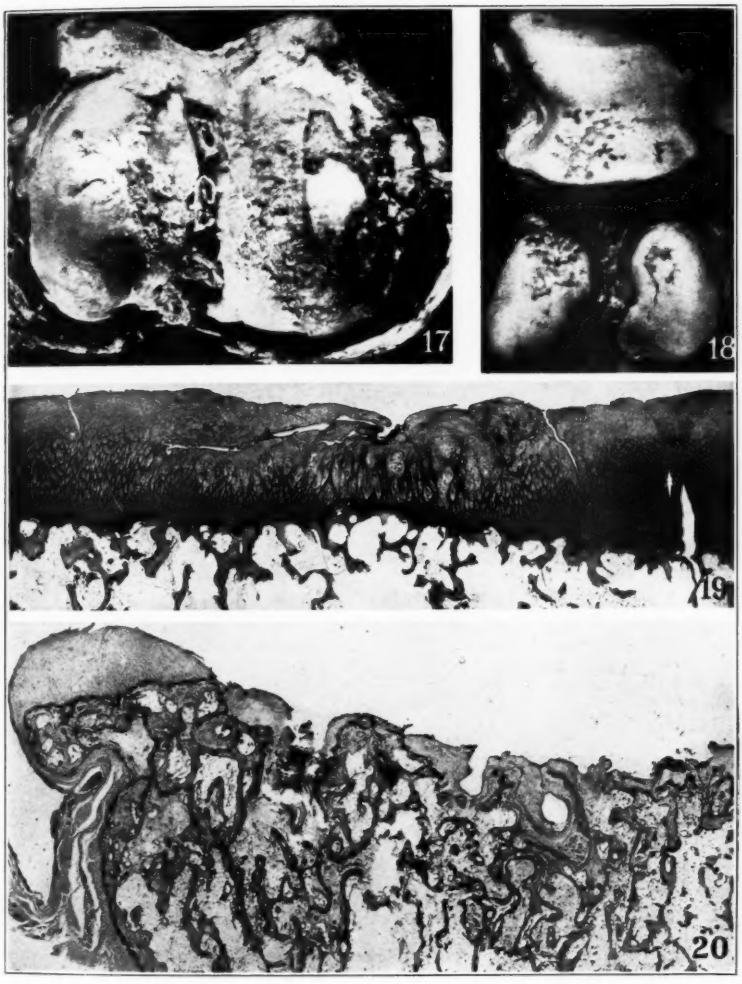

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PLATE 52

FIG. 21. Advanced lipping of patella fossa and condyle of human femur (see Fig. 17).

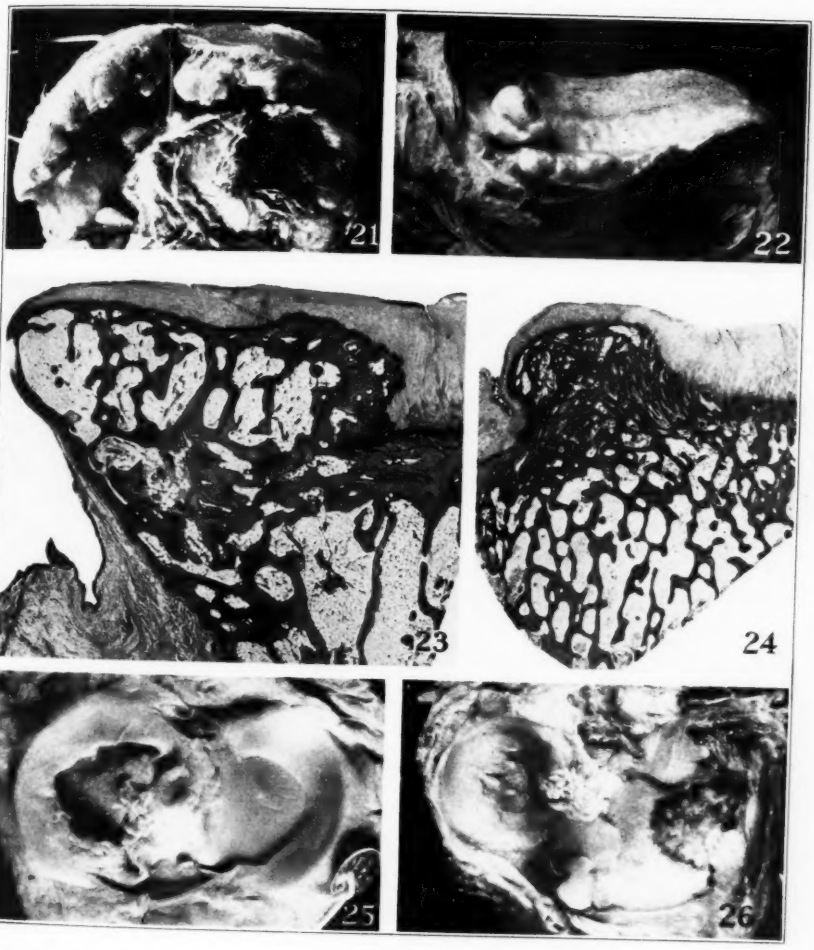
FIG. 22. Shelf formation on equine patella. Note pedunculated mass arising from bone margin. Fringed erosion occupies upper half of joint surface.

FIG. 23. Section of lipped margin of human patella fossa which grossly was less advanced than that shown in Fig. 21. New bone, which appears normal, rises above the old cortex and is covered by thin fibrillated cartilage. There was extensive deep ulceration of patella and patella fossa. Note extension on to old cortex.

FIG. 24. Section from the opposite side of bone of Fig. 22, showing cross section of the lip or shelf. Note similarity of Figs. 23 and 24.

FIG. 25. Human tibia. Mesial semilunar cartilage (left) shows fibrillation for a short distance from its attachment and a small fringe. Lateral meniscus essentially normal, early "blister" formation in cartilage on right.

FIG. 26. Human tibia. Lateral meniscus shows rather large pedunculated masses extended onto the eroded joint cartilage. Medial meniscus largely destroyed. Mass of bone covered with cartilage at lower center which extended to cervical ligament.



Callender and Kelser

Degenerative Arthritis

STUDIES ON EXPERIMENTAL RICKETS IN RATS*

II. The Healing Process in the Head of the Tibia and Other Bones

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Introduction

During the decade and a half in which serious experimental work on rickets has been carried on by the use of rats and other animals, remarkably little attention has been given to the study of the pathological details in the bones, except so much as has been necessary for diagnostic purposes. In an earlier paper (1934) we presented an account of the outstanding structural changes observed in bones during the active phase of rickets, with special reference to the behavior of the epiphyseal cartilage in the head of the tibia. In this paper we present a comprehensive account of the healing process.

The best account we have seen of the healing process in experimental animals is that by Pappenheimer (1922) on the healing of the ribs of rats under the influence of cod liver oil. Our findings are in complete keeping with his, but we have carried the study to greater detail than he did. From a study of numerous published pictures of bones of rats showing healing of rickets, mostly at low magnifications, we conclude that there is nothing unusual in our material. We present our account with confidence in its wide applicability.

Material and Methods

Number of Rats and Diets Employed

The observations recorded in the present paper are based on the study of 125 albino rats as follows: (1) normal controls fed a balanced diet, 26 rats; (2) various stages of active rickets, 31 rats; and (3) rickets in various healing stages, 68 rats.

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In each instance the rachitic condition was produced by the Steenbock-Black rachitogenic diet with high calcium, low phosphorus and deficient vitamin D.

Healing in the 68 rats was induced as follows: (1) by irradiated ergosterol (Viosterol), 0.00001 mg. daily for 5 days, 39 rats; (2) cod liver oil, 2 drops 3 times a week during experiment, 16 rats; (3) return to balanced diet, 3 rats; and (4) spontaneous healing, 10 rats. In all four groups the course of healing was the same, though there were differences in rate and permanence.

The young rats were fed the rachitogenic diet at the age of 4 weeks. Rickets was usually well developed by the age of 8 weeks. At the age of 8 to 9 weeks the feeding of the curative agent was begun. A few rats were carried as far as the age of 13 weeks, 5 weeks after the beginning of the curative regimen. The material includes rats under experimental handling at all seasons of the year beginning with the autumn of 1931 and ending with the spring of 1935.

Methods of Study

X-ray photographs were taken of all rats under light ether anesthesia at the time of sacrifice, and of most of those in the healing series at intervals of a week or less.

Bones were fixed in Bouin's fluid, usually at body temperature. Decalcification was in Müller's fluid (in which the potassium dichromate is the essential ingredient). Bones thus decalcified give a strong staining differentiation between calcified and non-calcified areas, which is lacking with other decalcifying agents. The stains used were Delafield's, Ehrlich's, or Harris' hematoxylin, usually followed by eosin. From the head of the tibia and the distal end of the femur of 35 rats we made a silver nitrate preparation as in the commonly used "line test," a process that blackens deposits of calcium salts. For these 35 rats we have thus three records: (1) X-ray photographs at weekly or more frequent intervals; (2) stained microscopic sections; and (3) silver preparations. For all others we have two records: X-ray photographs and microscopic sections. We studied X-ray films of all the bones of the legs of all rats, and microscopic sections from the distal end of the femur and of the radius and ulna, but our main study was on the head of the tibia. This bone was selected because of its

good size, rapid growth and general suitability, and because it is one of the bones of the leg which carries the weight of the animal. Moreover, there is special need for knowledge of its structure, because the head of the tibia has come more and more to be used by students of rickets in measuring the progress of healing.

The Identification of Calcified Areas

Each of the three methods we have employed gives useful information concerning the extent of calcification.

X-Ray Photographs: X-ray photographs portray a shadow of the total mineral salts, mostly of calcium, but with very little structural detail in bones as small as those of rats. Nor can X-ray photographs be advantageously studied under magnifications of more than a very few diameters (Fig. 1).

Silver Nitrate Preparations: The silver preparations show calcified areas with some detail, and may with profit be studied and photographed under magnifications of several diameters. Figures 5 to 10 show photographs of bones prepared in this way, the surface shown being made by splitting the head of the bone longitudinally.

Hematoxylin and Eosin Stain: It has been customary for a good many years to interpret a certain deep hematoxylin stain as indicative of calcification in bone and cartilage, even though the tissue has been subjected to decalcification before staining. There are some, however, who question whether such staining is indicative of calcification. The value of much of the work on both healthy and diseased bones depends on the reliability of this reaction. We have made the following observations of differential staining, which we have come definitely to believe identifies calcified areas, the certainty in our minds growing from the constant agreement between the pictures presented by X-ray photographs, silver preparations, and hematoxylin-eosin sections.

Differential Staining in the Epiphyseal Cartilage: In the epiphyseal cartilage of normal bones, the part of the matrix that is generally considered to be without calcification has a light gray color, or at most a moderate purple cast. In the portions of the matrix considered to be calcified there is a very deep purple hematoxylin stain with no trace of eosin coloring (Fig. 21). The cartilage remnants in the interior of recent normal bone trabeculae

show the same deep stain (Fig. 23). In severe rickets there is none of this coloring in the epiphyseal cartilage but it is seen in the cartilage remnants in the interior of the bone trabeculae that were formed before the onset of rickets.

Differential Staining in Osseous Structures: In both normal and rachitic bones the osteoid areas stain a uniform red color by eosin with little or no trace of hematoxylin. When hematoxylin alone is used, such areas are pale gray (Figs. 2 and 3). In areas commonly believed to be calcified, both hematoxylin and eosin participate, producing a purple color with more red than in calcified cartilage. Over the greater part of such areas the coloring is uniform, showing no granular structure, but near the surface of rapidly growing normal trabeculae, and in rachitic bones, the coloring may be in the form of purple granules (Figs. 2 and 3).

Severity of Rickets at Beginning of Healing

In the present paper we describe the healing of only the severe form of rickets, such as used by numerous workers for the assay of antirachitic substances. We considered rickets to be severe enough for our purposes when X-ray photographs showed the uncalcified epiphyseal cartilage in the head of the tibia to have attained a thickness of 1.5 to 2 mm. (Fig. 1). This condition was usually attained at the age of 8 to 9 weeks. At the same time growth had become decidedly subnormal and the rats had fallen well below the normal weight for rats of their age.

The Healing Process in the Head of the Tibia

Scales for Measuring the Progress of Healing

The "Line Test": The "line test" of McCollum *et al.* (1922) has been widely used in estimating the rate of healing. The four stages in common use are well illustrated in the colored figures of Bills and McDonald (1926). Figures 5 to 10 show the progress of calcification by this method.

X-Ray Scales: The use of the X-ray has also gained some vogue and has many advantages, though also some limitations. An elaborate X-ray scale has been devised by Bourdillon *et al.* (1931). It includes twelve stages which the authors have illustrated by an excellent series of reproductions. We have adopted it as that

against which we measure our microscopic and silver preparations. We have 639 X-ray photographs of the 125 rats in our series (as many as 17 of 1 rat), which we have grouped according to the twelve stages. We do not know whether so elaborate a scale as this is advantageous in the average bio-assay work, but we have found it exceedingly illuminating in our analysis of the healing process.

Our Interpretation of Bourdillon's Scale: We found it necessary to select a series of X-ray photographs from our own material to use as a standard, because Bourdillon used a straight anteroposterior view of the knee joint with the leg extended while we had been photographing our rats as the animal lay ventral side down under light ether anesthesia with the knee somewhat flexed. This position gave a more lateral view of the head of the tibia. We have made every effort to retain Bourdillon's intervals.

The Progress of Healing as Seen in X-Ray Photographs (Fig. 1)

Severe Rickets: No trace of calcification in cartilage region.

Stage 1: Faint diffuse shadow of new calcium deposit about midway between epiphyseal bone and end of shaft. Does not extend to posterior surface of bone. This new calcium deposit spreads toward the end of the shaft, as described in the following stages.

Stage 2: Shadow more dense, and at anterior margin of bone has come to form a narrow definite line.

Stage 3: Line shadow broader and extending half-way to posterior margin of bone. Diffuse shadow nearly to posterior margin.

Stage 4: Definite strong shadow extending from anterior to posterior surface of bone.

Stage 5: Shadow stronger and broader, and approaching end of shaft. Anterior margin of shadow forms sharp outline of surface of bone.

Stage 6: Shadow still broader and beginning to join end of shaft. Surface of bone well defined at both anterior and posterior margins of new calcium deposit.

Stage 7: New calcium, as shown by shadow, joined to end of shaft or very nearly so. Head of bone still has definitely rachitic shape as shown by well developed shoulder at end of shaft.

Stages 8, 9, 10, 11 and 12: In these stages the shape of the head undergoes correction by the gradual obliteration of the shoulder

at the end of the old shaft. During this process the shoulder recedes from the epiphyseal cartilage and at the same time becomes less prominent. At about Stage 9 the epiphyseal cartilage begins to grow thinner and by Stage 11 is about normal, though this is a point not clearly seen unless the plane of the photograph is just right. Stage 12 shows practically normal form and structure.

Parallel Between Line Test and X-Ray Stages: As nearly as we can tell by comparison with the figures of Bills and McDonald (1926) and others, and by study of our own silver preparations, the correspondence between the four line test stages and our twelve X-ray stages is about as shown in Table I.

TABLE I

Comparison of Line Test and X-Ray Stages

Line test stages		X-ray stages
+	equivalent to	1
++	" "	2
+++	" "	5 or 6
++++	" "	about 8

The General Nature of the Healing Process

In the study of healing it is important to keep in mind at all times the two main structural elements involved, *i.e.* the marrow and the solid tissues (bone and cartilage). It must be remembered also that both bone and cartilage have two constituent parts — the cells and the intervening matrix. The matrix in turn has two main parts — the organic portion and the calcium salts. The progress of calcification during healing is shown in the X-ray photographs just described and serves as a background for detailed study of structural changes. The marrow must be recognized as the vehicle through which the cartilage and bone are in large part nourished; by which they are built up and destroyed; and through which calcium salts are supplied and removed.

In our observations, healing began in the metaphysis close to the thin part of the epiphyseal cartilage, whence it quickly spread laterally into the projecting masses of thick cartilage at about the same level. From this beginning the reorganization spread toward the shaft in both the area of the metaphysis and the cartilage masses (Figs. 1, 6, 7 and 8). While this conspicuous progressive

reorganization was going on there was also taking place a quite generally distributed phase of healing.

The healing process may conveniently be described under the following headings: (1) the rachitic metaphysis; (2) the epiphyseal cartilage; (3) the diaphysis (shaft); (4) the epiphyseal center; (5) correction of shape; and (6) the marrow. Changes that are going on at the same time must of necessity be described consecutively.

Though the general structure of rachitic bones is familiar to most readers, it will be necessary to include in the account of healing short notes on certain features of rachitic bones.

The Reorganization of the Rachitic Metaphysis

The Rachitic Metaphysis: The rachitic metaphysis is a characteristic and important feature of rachitic bones which has received very little attention in papers dealing with rickets. It takes the place of the portions of the thickened cartilage removed during rickets (Figs. 5 and 11). It corresponds to the primary spongiosa of normal bones, but lack of calcification causes its structure to be quite different. It consists of cartilage remnants including both cells and matrix, upon which osteoid has been deposited in considerable amounts (Fig. 17). The cartilage-osteoid trabeculae of this region have the form of rather irregular masses, with a tendency to longitudinal orientation. Though these trabeculae in general appearance resemble osteoid rather than cartilage, the uncalcified cartilage matrix persists within them, and is destined to emerge during healing and to dominate the form and orientation of the trabeculae in the restored bone, as will be pointed out later. The marrow spaces, as seen in sections, are about equal in area to the trabeculae. In no case of severe rickets did we observe any calcification in the rachitic metaphysis.

The Cartilage-Metaphysis Junction: An important region in the consideration of healing is the border zone of the metaphysis, where it joins the thinner portions of the epiphyseal cartilage, because it is there that the marrow has made its greatest advance into the cartilage, and it is there that we have observed the earliest healing changes. Throughout the entire border area there were at intervals narrow projecting masses of cartilage which extended into the metaphysis where they became incased with osteoid, thus

making their contribution to the typical structure of the rachitic metaphysis (Fig. 17). Between these projecting cartilage masses were areas of vascular marrow which terminated against the cartilage. There the vascular channels often became considerably expanded, and there were frequently some erythrocytes outside their endothelial walls. In more advanced rickets, when cartilage removal had ceased, the vascular tissue at the cartilage border became largely replaced by cellular connective tissue, while in still more extreme cases the spaces had become largely filled with osteoid tissue (Fig. 17). The superficial region of a bone was likely to represent a more advanced condition than the interior.

The Beginning of Healing as Seen in X-Ray Photographs: In X-ray photographs the first indication of healing was, as generally recognized by students of rachitic bones, a faint shadow appearing in the uncalcified region about midway between the epiphysis and the end of the shaft (Fig. 1). It indicates the general location of the calcification but does not give precise information about the tissue elements involved. The first appearance of this shadow defines Stage 1.

The Beginning of Healing as Seen in Silver Nitrate Preparations: Silver nitrate preparations showed that this earliest calcification (the so-called "line") was in the metaphysis close to the edge of the cartilage (Fig. 6). It had the form of a flattened ring extending inward from the surface of the tibia from which beginning (Stage 1) calcification soon extended inward, until during Stage 2 the ring had become a complete plate (Fig. 7). This early spread of calcification was accomplished by invasion not only of the entire cartilage-metaphysis border zone, but also by extension into the projecting masses of the thick cartilage. While this spread was taking place the zone of calcification also grew thicker by extension into the metaphysis toward the shaft.

A few of our preparations showed a second, rather extensive area of calcification at early stages, deeper in the metaphysis, but this second area was not very common nor did it appear to play an important part in determining the course of healing. Figure 6 shows a bone with this calcified area (Ca 2), while Figure 7 illustrates the more common type which does not have it.

The Beginning of Healing as Seen in Stained Sections: In hematoxylin-eosin sections the first observed changes involved tissue

structure rather than calcification, *i.e.*, enlargement of the marrow spaces in the edge of the metaphysis close to the edge of the cartilage. This enlargement of spaces involved removal of osteoid, where it had clogged the spaces, and a loosening of the cellular structure where there had been dense masses of connective tissue cells. At the same time there took place a reduction in the size of the large vascular channels. The result was the early establishment of a more normal marrow in this region, composed of moderate sized vascular channels surrounded by connective tissue cells (*cf.* Figs. 17, 18 and 22). This early enlargement of the marrow spaces exposed short portions of cartilage trabeculae, connecting on the one hand with the general cartilage mass, and on the other with the cartilage-osteoid trabeculae of the metaphysis.

Earliest Calcification Seen in Stained Sections: We could not detect calcification quite as early in hematoxylin-eosin sections as in X-ray films and silver preparations. It was seen first in the exposed longitudinal cartilage trabeculae, in the edge of the metaphysis bordering the epiphyseal cartilage (Zone 1), and also very faintly in some of the osteoid masses of the adjacent region of the metaphysis (Zone 2). This calcified area corresponds in position to the earliest calcification seen in silver preparations (Fig. 6). In slightly more advanced healing stages (Stages 1 and 2) calcification became increasingly more evident in these regions (Figs. 4 and 18).

Though we have not in hematoxylin-eosin sections observed the very earliest calcification we have reason to believe that it occurs in the masses of osteoid which are very early removed in the first enlargement of marrow spaces close to the edge of the cartilage. This conclusion is supported by the observation that in all later stages the removal of osteoid was preceded by its calcification.

The reorganization of the entire metaphysis was accomplished essentially by a spreading of the processes whose beginnings have just been described.

Progressive Reorganization Processes: The spread of the healing process from the early "line" toward the shaft was carried on thus: calcification of the osteoid began in the metaphysis close to the edge of the cartilage, and progressed steadily toward the shaft, which it reached at about Stage 6. Before the calcified band had become very wide, there began the removal of the calcified osteoid

close to the cartilage. This removal process advanced steadily toward the shaft, always keeping some distance (a somewhat variable distance) behind the calcification front. As the osteoid became removed the exposed cartilage trabeculae became calcified, the calcification of cartilage trabeculae progressing toward the shaft as rapidly as did the removal of osteoid, but a short distance behind.

During the early stages of healing there were thus seen in the metaphysis three zones as follows: (1) adjacent to the epiphyseal cartilage a steadily widening zone characterized by numerous, slender, rather parallel, calcified cartilage trabeculae, with very little osteoid on them (Figs. 4, 7, 12, 18 and 19). (2) There followed a zone in which the masses of osteoid had undergone dilute calcification, but the contained cartilage trabeculae were not calcified. This zone was constantly moving toward the shaft. This zone is shown in silver preparations as irregular masses, in contrast with the slender parallel bars of Zone 1 (Fig. 7). It also shows clearly in stained sections (Figs. 4, 18 and 19). (3) Between the calcified osteoid area and the end of the shaft was an uncalcified zone, which grew continually narrower until it became obliterated when calcification reached the end of the shaft at about Stage 6 (Figs. 7 and 8).

General Reorganization Processes: While the progressive healing changes described above were going on, bringing about the widening of the zone of calcified cartilage trabeculae, there was also going on a less rapid removal of osteoid from the surface of trabeculae in the uncalcified zone. This produced a general reduction in size of trabeculae and enlargement of marrow spaces and caused the distinction between zones gradually to become less sharp, so that by Stage 5 or 6 there was no longer a clear-cut zonation (Figs. 8, 13 and 20).

The general arrangement of the trabeculae during these early stages of healing became increasingly more parallel and regular, the change being due to the removal of the osteoid, so that the parallel cartilage trabeculae, which had been largely obscured during rickets, came to dominate the pattern of the metaphysis as they do in normal bones. This result was made possible because the lacunae in the thickened cartilage had largely maintained their columnar arrangement, even through temporary submergence in

the rachitic metaphysis. Had the cell columns been greatly distorted the attainment of a normal pattern of trabeculae would have been much delayed.

The Union of the New Deposit of Calcium with the End of the Shaft: It will be noted that at Stage 6 (Fig. 1) the X-ray films do not show that the new calcium deposit had united with the end of the shaft, while silver and hematoxylin-eosin sections show that such union had taken place. The answer to the apparent discrepancy seems to lie in the fact that in the metaphysis near the end of the shaft, during Stages 5 and 6 the trabeculae were rather few and the marrow spaces large so that the total calcium at this level was not enough to throw a dense shadow (Figs. 8 and 13).

The later reorganization of the metaphysis is described along with the restoration of the epiphyseal cartilage.

The Reorganization of the Epiphyseal Cartilage

In considering the restoration of the epiphyseal cartilage to normal thickness and structure it is necessary to treat separately its two portions: (1) the thinner part or parts which have been reduced in thickness during the progress of rickets; and (2) the projecting masses which remain of full thickness, still resting upon the end of the shaft (Figs. 5 and 11). For details see our earlier paper (1934).

Removal of the Thick Parts of the Epiphyseal Cartilage: The thick projecting parts of the cartilage were all removed before there was any general reduction of the thinner portions. Removal of these portions was brought about by lateral erosion from the metaphysis, not by frontal attack from the end of the shaft. The removal progressed in such a manner that the last portions to be removed were usually isolated masses near the end of the shaft (Figs. 8 and 13).

The first step in the process of removal was the lateral spread of calcification, beginning at about Stage 2, from the early calcification area into these projecting masses (Figs. 6 and 7). This calcification soon extended clear across, and later spread toward the shaft.

Following its calcification the cartilage was promptly invaded by marrow masses, which usually entered from the side and later proceeded in a more longitudinal direction toward the shaft. This

invading marrow removed most of the uncalcified transverse walls between lacunae in the columns, and also some of the calcified longitudinal walls between columns of cells, leaving the usual, rather parallel pattern of calcified cartilage trabeculae. The fact that the columns of cells in the thickened cartilage were maintained without much distortion made possible this parallel arrangement. Upon these trabeculae, osteoid was promptly deposited in about the amount characteristic of normal bone trabeculae.

The removal of the projecting cartilage masses was largely completed by Stage 6, and entirely so by Stage 7 or 8, at which time the entire cartilage had become reduced to a rather uniform thickness of about double its normal measure (Figs. 13 and 14). With the removal of the projecting cartilage masses, the entire edge of the cartilage and the marrow adjacent to it assumed a rather normal appearance except that calcification extended inward only one or two cells (Fig. 22) instead of the normal extent of three or four (Fig. 21).

General Reduction of the Cartilage in Thickness: At about Stage 9 the general advance of the marrow into the cartilage first began, as shown both by microscopic observations of the removal front, and by measurable reduction in the thickness of the cartilage. At about Stage 10 calcification first extended into the cartilage to about its normal distance of three or four cells. At this time the number of fully enlarged cells in the cartilage columns was still considerably in excess of the normal number. By Stage 11 or 12 the cartilage was reduced to its normal thickness of about 350 μ (sometimes less) and at the same time the number of hypertrophied cells was reduced to the normal number of three or four (Figs. 15 and 21).

Multiplication of Cartilage Cells: In a previous paper (1934), we recorded our observations that the cells of the epiphyseal cartilage ceased multiplication and the bone ceased to elongate after rickets became severe. We have also observed that during healing the bone did not again begin to elongate until about the close of the healing process, at which time the epiphyseal cartilage had assumed normal structure. We have not made careful search for mitotic figures at the completion of healing, but cell division doubtless was resumed at that time inasmuch as the bone began to elongate.

The Cartilage-Metaphysis Area During the Later Stages of Healing

The trabeculae produced by the reorganization of the thick portions of the cartilage resembled very closely in shape and attitude those formed in the adjacent metaphysis. Both areas contributed to the building of a new end for the shaft, terminating against the restored epiphyseal cartilage.

The later phase (Stages 7 to 12) of the reconstruction of this region involved increasing calcification of the cartilage and osteoid in the trabeculae. At Stage 6 there was very little calcification in the osteoid on these trabeculae, though in this respect there was considerable individual variation (Fig. 22). Beginning with Stage 9 there was usually complete dense calcification, though in some rats the attainment of this condition was delayed until Stage 12. There was also continued, though not radical modification of the form and attitude of the trabeculae, brought about by local removal and addition of osseous material. The result was that the structure in this region grew more and more normal (Figs. 13–16).

The Parallelizing of the Divergent Bone Trabeculae: Throughout the later healing stages there persisted one feature which marked the newly reorganized spongiosa as having come through a rachitic period, *i.e.*, the considerable radial divergence of the trabeculae, a direct result of the characteristic divergence of the cells columns in the rachitic cartilage, in contrast with the parallel arrangement in normal bones (Dodds and Cameron, 1934). The fact that this divergence of the trabeculae emerged in the late healing stages, after long submergence in the uncalcified rachitic metaphysis, is further striking evidence of the dominance of the cartilage pattern in determining the form and attitude the bony trabeculae assume. Figure 14 shows this divergence clearly at Stage 8. Figure 15 illustrates a late stage in the correction of this defect, while Figure 16 shows the parallel arrangement in a normal bone.

The Reorganization of the End of the Shaft

During severe rickets, the end of the shaft was clearly differentiated from the cartilage-metaphysis region because of the persistence of calcification in the pre-rachitic cartilage-bone trabeculae

in the shaft (Figs. 5 and 11). As might be expected, these trabeculae became less robust and more numerous toward the end of the shaft, just as they do in normal bones, but in severe rickets we found them to be much distorted, smaller, and less numerous than in normal bones (*cf.* Figs. 11 and 16). This condition explains the blurring commonly observed in the end of the rachitic shaft in X-ray photographs. Upon these calcified remnants there had been deposited much osteoid, sufficient to reduce greatly the size of the marrow spaces. The end of the shaft in all of our bones had the typical concave or "cupped" form which characterizes rachitic bones.

No special study was given to the cortical bone of the shaft, except to observe that in advanced rickets there was a thick layer of osteoid on both its outer and inner surfaces and that its calcified portion had become somewhat reduced in thickness.

During the first few stages of healing the spongiosa of the shaft with its remnants of pre-rachitic calcification was undergoing reorganization at the same time as the adjacent metaphysis, and there was a general similarity between the changes in the two regions. The osteoid, which had become very abundant during severe rickets, began very early in the healing process to be reduced in amount, its removal about keeping pace with the general reduction in the adjacent part of the metaphysis (Figs. 11, 12 and 13).

As in the metaphysis, the removal of osteoid in the end of the shaft was preceded by a dilute granular calcification which began at about Stage 2.

There also persisted a certain residue of osteoid on the calcified pre-rachitic trabeculae, in which, at about Stage 6, there began the formation of more dense deposits of calcium salts, producing an addition to the trabeculae by which the amount of bone was definitely increased (Figs. 13 and 14). These trabeculae at Stages 7 and 8 became quite robust, in contrast with the more slender ones in the adjacent metaphysis, and by them the end of the pre-rachitic shaft may be located (Fig. 14).

During the few later stages of healing, continued adjustment of trabeculae caused this difference to disappear, so that the boundary line between the pre-rachitic shaft and the rachitic metaphysis could no longer be recognized (Figs. 14 and 15). Thus the re-

organized tissues of the metaphysis-cartilage region have developed into a new end of the shaft, which terminates in a normal manner against the restored epiphyseal cartilage (Text-Fig. 1 B and C).

The Correction of Cupping: As the epiphyseal cartilage became restored to normal thickness and structure, the new end of the shaft assumed very much the normal form, having a definite convexity at the margin. At the same time the old concave termination of the shaft had been obliterated by its union with the metaphysis, as described above. Thus cupping was corrected by the substitution of a new end of the shaft for the old one, as seen in Text-Fig. 1 B and C and Figs. 7 to 9, and 13 to 15.

The Reorganization of the Epiphyseal Center

The epiphyseal portion of the bone was only mildly affected by rickets. The thickness of the trabeculae was only slightly increased, and there was only a thin layer of osteoid, not over 25 μ in thickness, formed upon them. The total amount of calcified material was definitely decreased, as shown by both silver and hematoxylin preparations. The marrow was not apparently changed in structure (*cf.* Figs. 5 and 9, and 11 and 16).

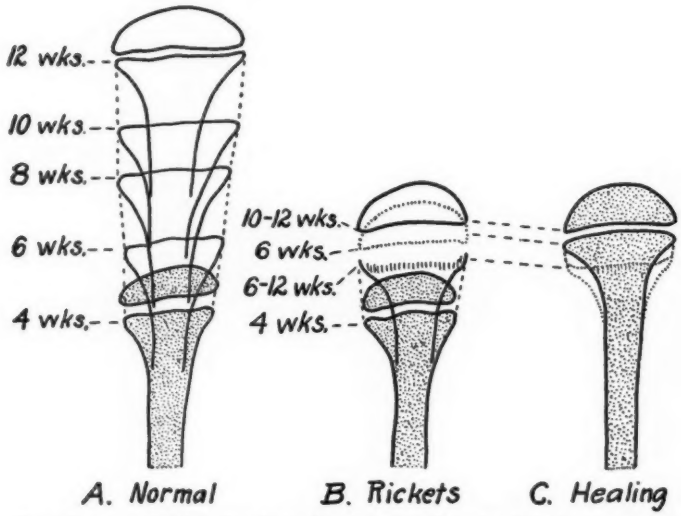
Healing in this region needs only brief mention. Calcification began to appear in the osteoid of the trabeculae in Stage 2. At about Stage 7 the amount of osteoid began to decrease and by Stage 11 the trabeculae appeared normal.

The Correction of the Shape of Rachitic Bones

Tubulation: Inasmuch as the ends of the diaphysis adjacent to the epiphyseal cartilages are greatly expanded in normal bones, it is necessary during the elongation of bones that there be a constant removal of recently formed bone from this region if the bone is to maintain its proper form. This process has been called "tubulation" by Jansen (Harris, 1933). The need for this removal becomes evident when we superpose X-ray photographs or scale drawings showing successive stages of a growing bone (Text-Fig. 1 A).

The Mechanism of Normal Tubulation: Our observations on the bones of healthy young rats show clearly that in the expanding curved portion of the end of the diaphysis, just below the epi-

physeal cartilage, there is no cortical layer of bone, but the parallel trabeculae end freely in the periosteum (Figs. 16 and 23). On the ends of these trabeculae there are many large osteoclasts. It is evident that by the continued removal of bone from the ends of



TEXT-FIG. 1. Outline drawings showing growth and tubulation of the normal and rachitic tibia from age 4 weeks to 12 weeks. Based on X-ray photographs enlarged to scale.

A = normal tibia, showing positions of end of shaft at biweekly intervals. The broken line bounds the area which must be removed in the normal process of tubulation.

B = rachitic tibia, showing limited growth during severe rickets. The shaft ceased to elongate after about 2 weeks on the rachitogenic diet (at age 6 or 7 weeks), due to cessation of normal cartilage removal. After this time the bone continued to elongate up to about 10 weeks by continued thickening of the epiphyseal cartilage. Tubulation ceased at about 6 weeks.

C = healing tibia, showing condition at end of healing process but before bone has resumed growth. New end of shaft has been formed by reorganization of thickened cartilage and rachitic metaphysis. Dotted lines bound areas removed in correcting the shape of the bone and the reëstablishment of tubulation.

these trabeculae the characteristic curvature of this region of the shaft may be maintained and the tubular form of the shaft assured.

Interference with Tubulation During Rickets: During the development of rickets, osteoid was deposited in a thick layer under the periosteum overlying the free ends of the trabeculae in the

curved region of the shaft adjacent to the epiphyseal cartilage (Figs. 5, 11 and 24). At the same time the osteoclasts disappeared from the ends of the trabeculae. Thus tubulation ceased and the accumulation of osteoid added to the bulk of the bone in this region.

The Form of the Head of the Rachitic Bones: The rachitic bones continued to elongate for some time after the cessation of tubulation, the elongation being due to the continued thickening of the epiphyseal cartilage, even though none of it became incorporated in the end of the shaft proper (Text-Fig. 1 B). The result was that the portion added to the length of the bone after the cessation of tubulation had a rather uniform thickness equivalent to that at the level of the epiphyseal cartilage, or in some bones a gently tapered form, due to moderate increase in the diameter of the epiphyseal cartilage even during severe rickets (Text-Fig. 1 B, and Figs. 1, 5 and 11).

We have made numerous measurements on X-ray films of the bones at the wrist and knee and find no enlargement of the calcified structures in rachitic rats at either place. Nor do microscopic sections show any excessive amounts of uncalcified tissues at joints. From these observations we conclude that the unusual prominence of the joints in living rachitic rats is due to an undersized condition of the limbs adjacent to the joints rather than an enlargement of the joint.

The Correction of the Rachitic Form: At about Stage 2 there began to appear clouds of calcium granules in the osteoid covering the ends of the trabeculae in the curvature of the end of the shaft, just as similar deposits were forming in osteoid throughout the metaphysis and end of the shaft. At about Stage 5 the removal of this osteoid was under way, and by Stage 8 it had been completed (*cf.* Figs. 13 and 14). After the removal of the osteoid, osteoclasts reappeared upon the calcified trabeculae and the correction of shape then progressed steadily by the removal of calcified material (Figs. 1, 14 and 15). In our scale of healing Stage 12 has been defined as that at which the form of the bone no longer shows the rachitic deformity in X-ray photographs. It is of interest to note that by the time the external form had been corrected, the reorganization of the internal structure had also been brought to practical completion.

The Reorganization of the Marrow

The Marrow in Healthy Bones: In general there are four main functions performed by marrow: (1) osteogenesis, (2) hematogenesis, (3) conduction, and (4) storage, with definite structural components for each function. In the bones of healthy young rats the osteogenetic marrow is found conspicuously in the spongiosa of the shaft adjacent to the epiphyseal cartilage, but it occurs also on surfaces of bone trabeculae everywhere. Its various structural elements include conspicuously the osteoblasts and the osteoclasts. It is concerned with the removal of the epiphyseal cartilage and with the deposition and removal of bone tissue. The periosteum shares in the osteogenetic function.

The hematogenetic marrow in the tibia of young rats extends to within 300 to 600 μ of the epiphyseal cartilage, occupying the medullary cavity and the larger spaces in the spongiosa. The conducting function is accomplished by the abundant vascular channels, the walls of which are composed of little more than endothelium. In young rats the storage function is represented by only a few adipose cells.

The Marrow During Rickets: Though there is considerable variation in amount and nature of marrow during rickets, the following statements, we believe, express average conditions fairly well.

When rickets had developed to the extent that osteoid instead of bone was being deposited upon the trabeculae, the removal of material from the trabeculae ceased, or at least deposition was greatly in excess of removal. At this time osteoclasts nearly disappeared but numerous osteoblasts continued actively to deposit osteoid. Thus the trabeculae became greatly enlarged and the marrow spaces correspondingly reduced in size. Finally the osteoblasts and most of the undifferentiated marrow cells disappeared, and little was left of the marrow but endothelial vascular channels whose walls were nearly or quite in contact with the osteoid walls within which they lay (Fig. 17). There thus remained scarcely more than the conducting elements of the marrow. This condition was observed both in the metaphysis and in the adjacent end of the shaft.

During the course of rickets the hematogenetic marrow underwent little apparent change, an observation in harmony with the

findings of Sure and Kik (1931) to the effect that rickets produced no noteworthy interference with the process of hemopoiesis. In severe rickets this type of marrow approached rather closely to the end of the shaft, but did not enter the rachitic metaphysis.

The Marrow During the Healing of Rickets: Early in the healing process there appeared numerous large osteoclasts, most abundantly near the cartilage-metaphysis junction, but also generally distributed throughout the metaphysis and the spongiosa of the shaft. They appeared in the scant connective tissue between the endothelial blood channels and the osteoid. In some rats we have seen a considerable abundance of osteoclasts, apparently removing osteoid, even before there was any evidence of calcification. Osteoclasts continued to be very abundant through Stages 2 and 3, but by Stage 4 there was a decrease in their number. By that time the marrow spaces throughout the metaphysis and in the end of the shaft had become considerably larger and the amount of connective tissue surrounding the vascular channels had increased (Figs. 4, 17, 18 and 22).

During the early reorganization of the metaphysis, osteoclasts were especially abundant in the zone of calcified osteoid, the zone where the removal of osteoid was going on most rapidly. When the epiphyseal cartilage began to undergo rapid reduction in thickness, at about Stage 9, osteoclasts became abundant upon it, where they occupied characteristic positions on the ends of projecting calcified trabeculae.

The cellular elements responsible for the deposition phase of osteogenesis, the osteoblasts, first become common at about Stage 2 in Zone 1, the marrow zone of calcified cartilage trabeculae (Figs. 18 and 22). With the widening of this zone, osteoblasts spread throughout the metaphysis, and at the same time become abundant in the areas reclaimed from the projecting cartilage masses, and in the shaft. Thus the constructive elements of the marrow become widely established.

Hematogenic marrow began to develop in the end of the shaft adjacent to the metaphysis during Stages 5 and 6. It appeared among the connective tissue cells surrounding the vascular channels of the larger marrow spaces. In the metaphysis during Stages 5 and 6 there were formed a number of excessively large marrow spaces (Fig. 13). In them the vascular channels became greatly

distended, with very little connective tissue between their endothelium and the surrounding osteoid. It was in the connective tissue of these large spaces, during Stages 5 to 7, that we first observed hematogenetic marrow in the metaphysis, at first in small amounts, but later increasing considerably, with a corresponding reduction in the size of the blood vessels. In the later stages of healing it advanced to its normal distance from the epiphyseal cartilage. At first it was in rather dilute form, and not until about Stage 12 did it attain normal density. Adipose cells appeared for the first time in this marrow at about Stage 12. So it was that by the time the bone and cartilage had attained normal form and structure, the marrow had also attained rather complete reorganization.

Observations on Other Bones

In the distal end of the femur the observed details were very much like those described for the tibia, though they were less easily studied on account of the convoluted form of the epiphyseal cartilage. The distal ends of the radius and ulna differed from the proximal end of the tibia in that during severe rickets the persisting thick cartilage usually had the form of several projecting tongues instead of one large mass. Healing began in the metaphysis as it did in the tibia and spread toward the shaft and also laterally into the projecting cartilage masses, and later toward the epiphysis into the thinner portion of the cartilage. The general picture during healing, as shown both by X-ray and by microscopic study, was essentially the same as in the head of the tibia.

General Résumé of the Healing Process

In the healing process of rachitic bones, as seen in the head of the tibia, there were two rather distinct phases. In the first phase, including Stages 1 to 6, there was accomplished a rather rough preliminary internal reorganization, involving in the main the removal of excess osteoid and important changes in the marrow. In the second phase, Stages 7 to 12, there took place a refinement of structural detail, and the correction of the shape of the bone, brought about in part by a continuation of certain of the processes initiated during the first phase.

The preliminary reorganization was accomplished largely in

TABLE II

General Sequence of Events in Healing

The two general phases of healing	Preliminary reorganization						Refinement of details					
Stages of healing as shown by X-ray photographs	1	2	3	4	5	6	7	8	9	10	11	12
Provisional calcification of osteoid in metaphysis	x	X	X	X	X	x	–	–	–	–	–	–
Calcification of cartilage trabeculae in metaphysis	x	X	X	X	X	X	–	–	–	–	–	–
Calcification of thick parts of epiphyseal cartilage	–	x	X	X	X	x	–	–	–	–	–	–
Removal of osteoid in metaphysis and shaft	x	X	X	X	X	x	–	–	–	–	–	–
General reorganization of osteogenetic marrow	x	X	X	X	x	x	–	–	–	–	–	–
Osteoclasts abundant in metaphysis and shaft	x	X	X	x	:	:	:	:	:	:	:	:
Trabeculae rapidly changing shape and attitude	:	x	X	X	X	x	–	–	–	–	–	–
Trabeculae undergoing continued but slower correction	–	–	–	–	–	–	x	x	x	x	x	x
Hematogenetic marrow developing in metaphysis	–	–	–	–	–	:	x	X	X	X	X	X
Osteoid being removed from expanded surface of shaft	–	–	–	–	:	x	X	X	–	–	–	–
Shape of bone being corrected by removal of trabeculae	–	–	–	–	–	–	–	:	X	X	X	:
End of pre-rachitic shaft becomes indistinguishable	–	–	–	–	–	–	–	:	X	X	–	–
Permanent dense calcification of bone trabeculae	–	–	–	–	–	–	–	:	:	x	x	x
Calcification zone of epiphyseal cartilage established	–	–	–	–	–	–	–	–	:	x	x	x
Normal removal of epiphyseal cartilage resumed	–	–	–	–	–	–	–	–	:	x	x	x
Epiphyseal cartilage restored to normal thickness	–	–	–	–	–	–	–	–	–	–	x	x
Mitosis in epiphyseal cartilage probably resumed	–	–	–	–	–	–	–	–	–	–	–	x
Cupping of end of shaft becomes corrected	–	–	–	–	–	–	–	–	–	:	x	x
Divergence of bone trabeculae becomes corrected	–	–	–	–	–	–	–	–	–	–	x	x

X = rapid progress of reorganization; x = less rapid progress; : = slow progress.

reverse direction, that is progressing from the cartilage toward the shaft, whereas in the second phase the direction of progress was again restored to normal, that is from the shaft toward the epiphysis. The general progress of healing is shown in Table II.

The Normality of our Healed Rachitic Bones: Comparison of Figures 15 and 16 shows how close may be the resemblance between a rachitic bone after restoration by the use of irradiated ergosterol or cod liver oil and one from a rat that never had rickets. Such excellent restoration is typical of our results, though in most such cases it seemed that calcification hardly came up to normal.

The Calcification Pattern During Healing: It has frequently been stated (Hess, 1929; Harris, 1933, and others) that in the healing of rickets calcification begins in the epiphyseal cartilage at the level where it would have been had rickets not developed. Our observations do not support this view. Had rickets not developed, calcification would have extended into the cartilage to include the whole zone of hypertrophied cells. We have always observed calcification to begin in the metaphysis adjacent to the cartilage, and not until well toward the completion of healing did it reach the level in the cartilage where it would have been had rickets not developed.

It is of interest to note also, that, as generally observed, calcification at first spreads toward the shaft in a direction opposite to that followed in healthy bones, and only in later healing stages is the normal direction resumed. The cause for this peculiar gradient of calcifying power is not evident. It is also of interest that the same order is followed by calcification of excised rat bones *in vitro*, as shown by numerous published illustrations.

Summary

1. Rickets was produced by the Steenbock-Block diet; healing was brought about mainly by the administration of irradiated ergosterol.

2. Healing is described in detail from study of hematoxylin-eosin sections, silver preparations, and frequent X-ray photographs.

3. The twelve X-ray stages of Bourdillon are used as a scale for measuring the progress of healing.

4. The first indication of healing was calcification in the rachitic

metaphysis close to the edge of the epiphyseal cartilage, whence it spread, first through the metaphysis toward the shaft and later into the cartilage toward the end of the bone.

5. The preliminary reorganization of the metaphysis involved: provisional dilute calcification of the osteoid; calcification of the exposed cartilage trabeculae; increase in amount of marrow; and its restoration to normal structure. These changes restored a somewhat normal configuration of trabeculae by exposing the rather parallel cartilage trabeculae. The end of the shaft undergoes much the same changes.

6. At the same time the projecting cartilage masses became calcified and were partly removed, leaving calcified trabeculae very similar to those in the metaphysis.

7. During the latter half of healing the shape of the bone became corrected; hematogenetic marrow formed in the new end of the shaft which had been formed from the metaphyseal region; cupping was corrected, the entire epiphyseal cartilage became normal in thickness and structure; and the reorganized trabeculae attained normal dense calcification.

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DESCRIPTION OF PLATES

Numerals, 1, 2, 3 (except in Fig. 1) indicate the three zones of the healing metaphysis as follows: 1 = zone of calcified cartilage trabeculae; 2 = zone of calcified osteoid; 3 = zone of uncalcified osteoid and cartilage.

Ca 1 = calcium deposit in edge of metaphysis, the "line" shown in silver preparations; Ca 2 = an unusual calcium deposit in metaphysis; C C = calcified cartilage trabeculae (in Zone 1); C O = calcified osteoid (in Zone 2); D = diaphysis or shaft; Ep = epiphyseal bone center; Ep C = epiphyseal cartilage; G = granular calcium deposit; H_1, H_2, H_3, etc., = healing stages; N = bone from normal rat; O = osteoid; Obl = osteoblasts; Ocl = osteoclasts; P = periosteum; R = bone from rat with acute rickets; U M = uncalcified rachitic metaphysis.

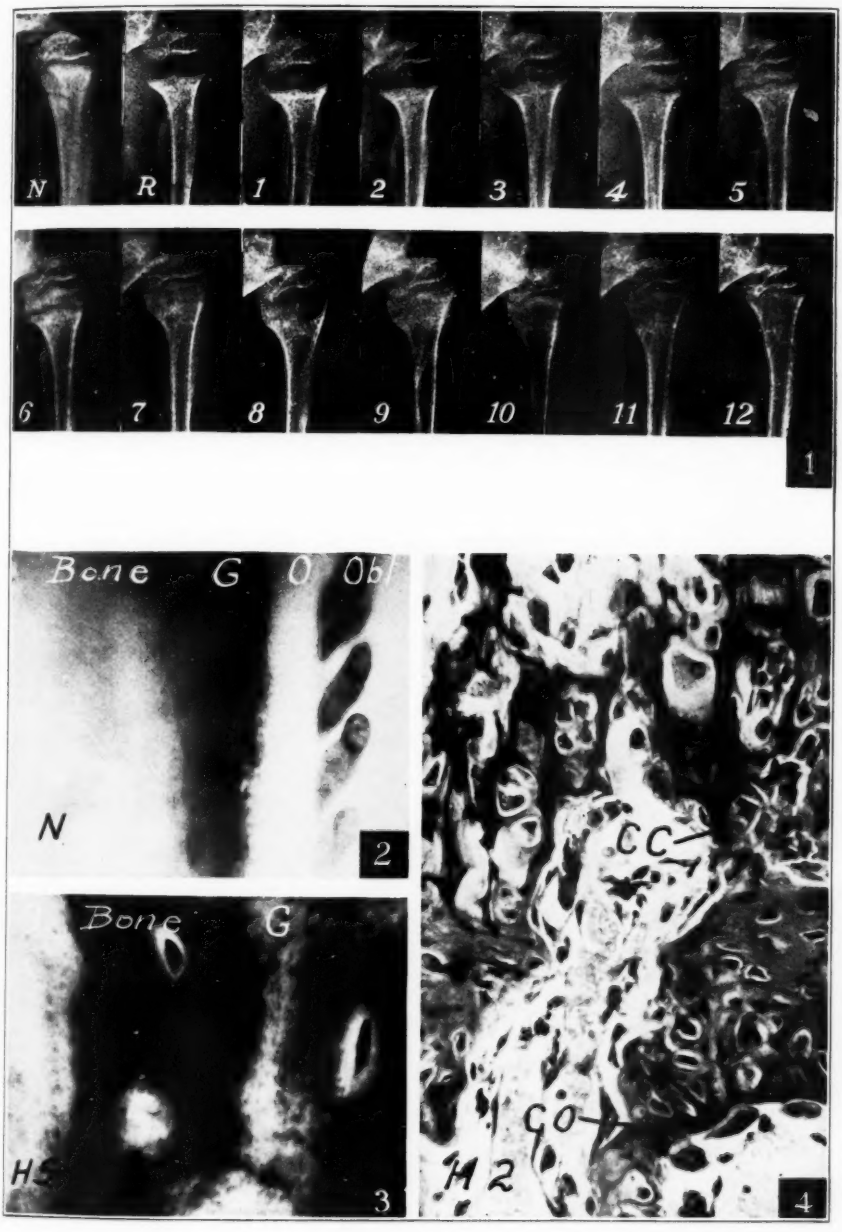
PLATE 53

FIG. 1. X-ray photographs of head of tibia showing our interpretation of the twelve healing stages of Bourdillon *et al.* Position of bones when rat lies relaxed under light ether anesthesia. N = normal bone; R = rachitic bone; 1, 2, 3, etc., the twelve healing stages. Reproduced from X-ray films. × 2.

FIG. 2. Vertical section through surface of normal bone trabecula which is growing rapidly. Shows osteoblasts (Obl) under which is a layer of osteoid (O) 8 μ thick. Beneath this a layer of granular calcium (G) which grows more dense with depth. This deeply stained layer blends into the general mass of bone which stains less deeply and does not show granular structure. × 900.

FIG. 3. Area in rachitic bone at Stage 5 of healing. Dark areas represent pre-rachitic bone, stained deeply with hematoxylin. Osteoid (O) is pale, and recently formed calcium granules (G) are shown in varying density. × 900.

FIG. 4. Area in metaphysis of bone at healing Stage 2. Zone 1 shows calcified cartilage trabeculae (C C), from which most of the osteoid has been removed. Zone 2 shows calcification in osteoid (C O) but none in the contained cartilage trabeculae. Shows nature of restored marrow clearly. × 450.



Dodds and Cameron Studies on Experimental Rickets in Rats. II

PLATE 54

FIGS. 5, 6, 7, 8. Head of tibia, silver preparations (line test). Calcified areas colored dark. × 15.

FIG. 5. Severe rickets, showing epiphyseal ossification (Ep), thick and thin portions of epiphyseal cartilage (Ep C); uncalcified rachitic metaphysis (U M); and diaphysis (D).

FIG. 6. Healing Stage 1. Structures as in Fig. 5, except for deposition of calcium line (Ca_1) in border of metaphysis, and a second rather unusual area of calcification (Ca_2) deeper in the metaphysis.

FIG. 7. Healing Stage 2. The calcified area has extended entirely across the bone, both in the metaphysis and in the thickened part of the cartilage. It has also become thicker by expansion toward the shaft. The three zones of the healing metaphysis are shown. Zone 1 of calcified cartilage trabeculae; Zone 2 of calcified osteoid masses; and Zone 3, the uncalcified portion.

FIG. 8. Healing Stage 6. Calcification has extended entirely across the metaphysis to the end of the shaft obliterating Zone 3. It has also invaded most of the thickened cartilage area. Zone 1 has become greatly widened, but is no longer sharply distinct from Zone 2. The trabeculae have attained approximately their normal configuration. The large uncalcified area in the center of the bone is unusual. Variations in pattern of healing are by no means uncommon. The form of the bone is still rachitic.

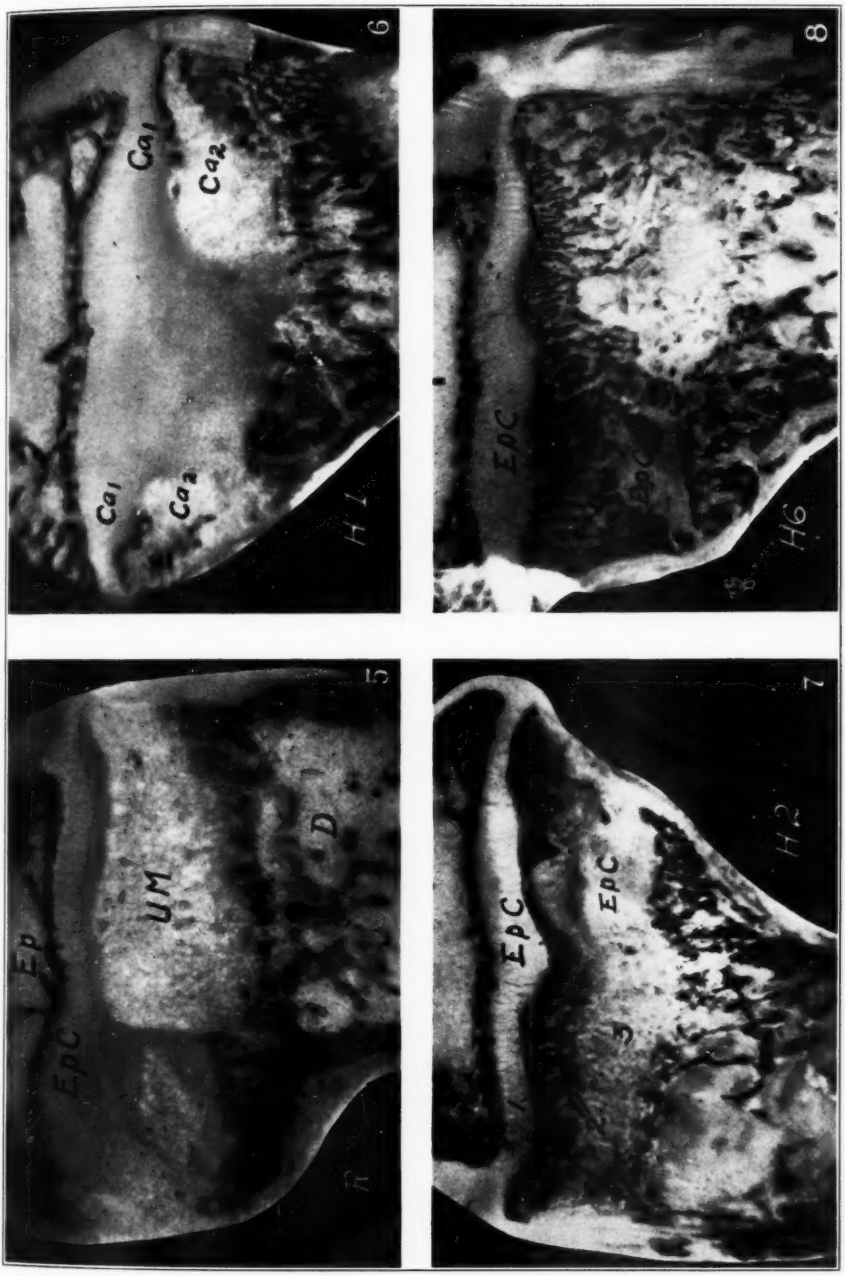


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PLATE 55

FIGS. 9, 10. Silver preparations of head of tibia, continuing the series begun on Plate 54. × 15.

FIG. 9. Healing Stage 12. The epiphyseal cartilage has been reduced to normal thickness and the entire pattern of calcification has progressed to about normal amount and distribution. Former rachitic metaphysis and end of pre-rachitic shaft have united to form new end of shaft with rather normal spongiosa. Cupping has been corrected, and shape of bone has become normal.

FIG. 10. Normal tibia, for comparison with healing Stage 12, shown in Fig. 9.

FIGS. 11, 12. Sections through head of tibia stained with hematoxylin and eosin. The dark color of the epiphyseal cartilage does not indicate calcification. The distinction between calcified and uncalcified cartilage is seen clearly under microscopic observation. × 15.

FIG. 11. Severe rickets (*cf.* Fig. 5). Epiphyseal cartilage (Ep C) showing reduced region, still abnormally thick, and the thickened area still resting upon the end of the pre-rachitic shaft. Note entire absence of calcification in the metaphysis (U M), and the abundance of osteoid and small amount of calcification in the end of the shaft (D). Note extreme rachitic form of the head of the bone.

FIG. 12. Healing Stage 2. The most conspicuous healing change is the development of the zone of calcified cartilage trabeculae (Zone 1) in the border of the metaphysis. Zones 2 and 3 are not as clearly shown as in the silver preparation (*cf.* Fig. 7).

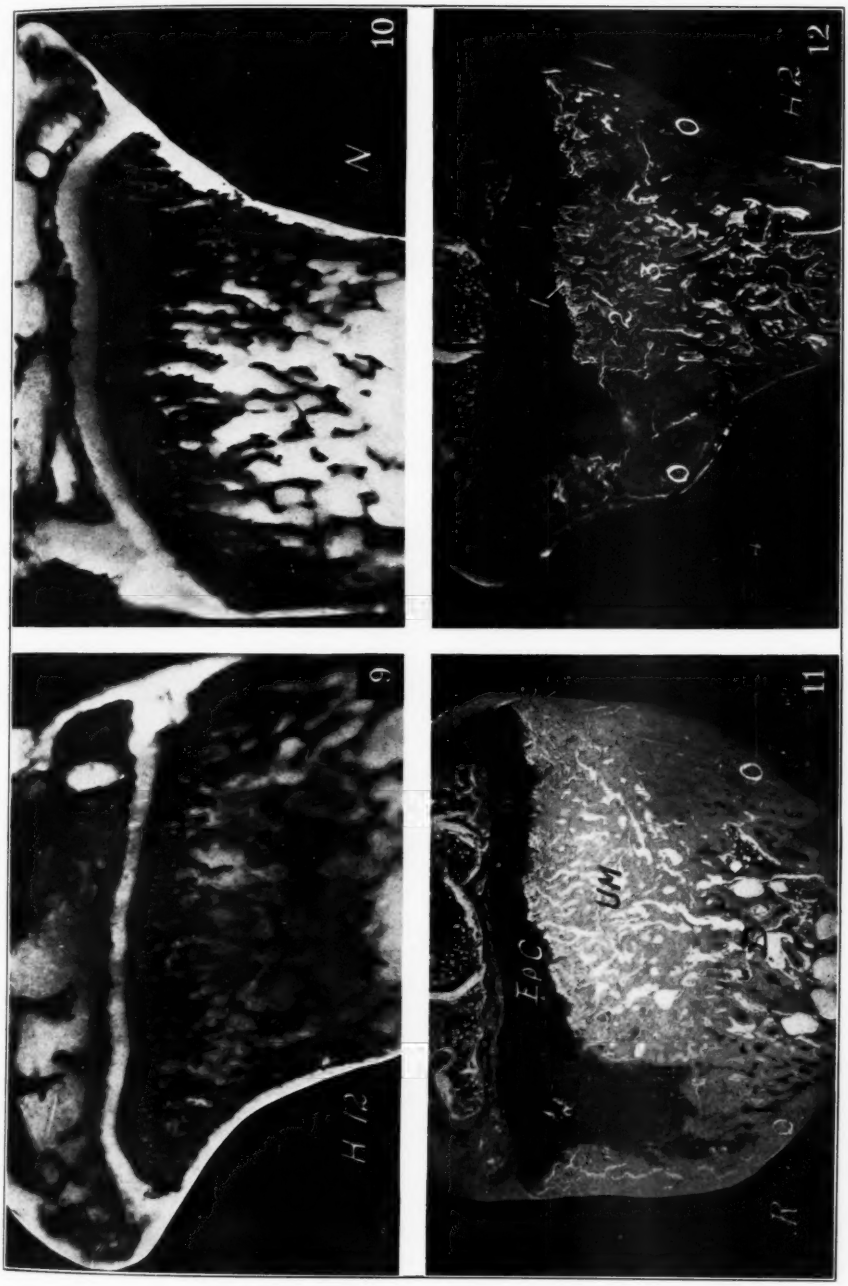

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Plate 56

Figs. 13, 14, 15, 16. Sections stained with hematoxylin and eosin. Continuation of series begun on Plate 55. × 15.

Fig. 13. Healing Stage 6 (*cf.* Fig. 8). Thick part of cartilage largely removed (represented only by isolated remnant adjacent to end of shaft). Trabeculae have become more parallel by removal of excess osteoid, allowing underlying pattern of cartilage to dominate. Zonation no longer distinct, due to spread of Zone 1 toward shaft. Dilute provisional calcification of osteoid has spread to end of shaft. Osteoid covering still present on curvature of head.

Fig. 14. Healing Stage 8. Thick part of cartilage completely removed, but entire remaining portion of cartilage is still abnormally thick. Trabeculae approaching normal configuration, though the radial divergence caused by a similar divergence of the cell columns in the thickened cartilage still shows plainly. Calcification in cartilage trabeculae is strong, but osteoid covering is still not fully calcified. Larger calcified trabeculae in end of shaft still differentiate it from metaphysis. Osteoid has been removed from surface of head but rachitic shape still persists. Portion to be removed in correction of shape outlined in broken lines. Hematogenetic marrow is developing in large marrow spaces in lower part of metaphysis.

Fig. 15. Healing Stage 12 (*cf.* Fig. 9). Epiphyseal cartilage reduced to normal thickness. Trabeculae have assumed nearly normal configuration and are well calcified. The radial divergence has been nearly corrected. Shaft and metaphysis are fully united. Cupping has been corrected and shape of bone restored to normal by reëstablishment of tubulation process.

Fig. 16. Normal bone, for comparison with Fig. 15. The lighter coloring of the epiphyseal cartilage in this figure is due not to a difference in the cartilage, but to the use of a different hematoxylin stain from that used in other sections.

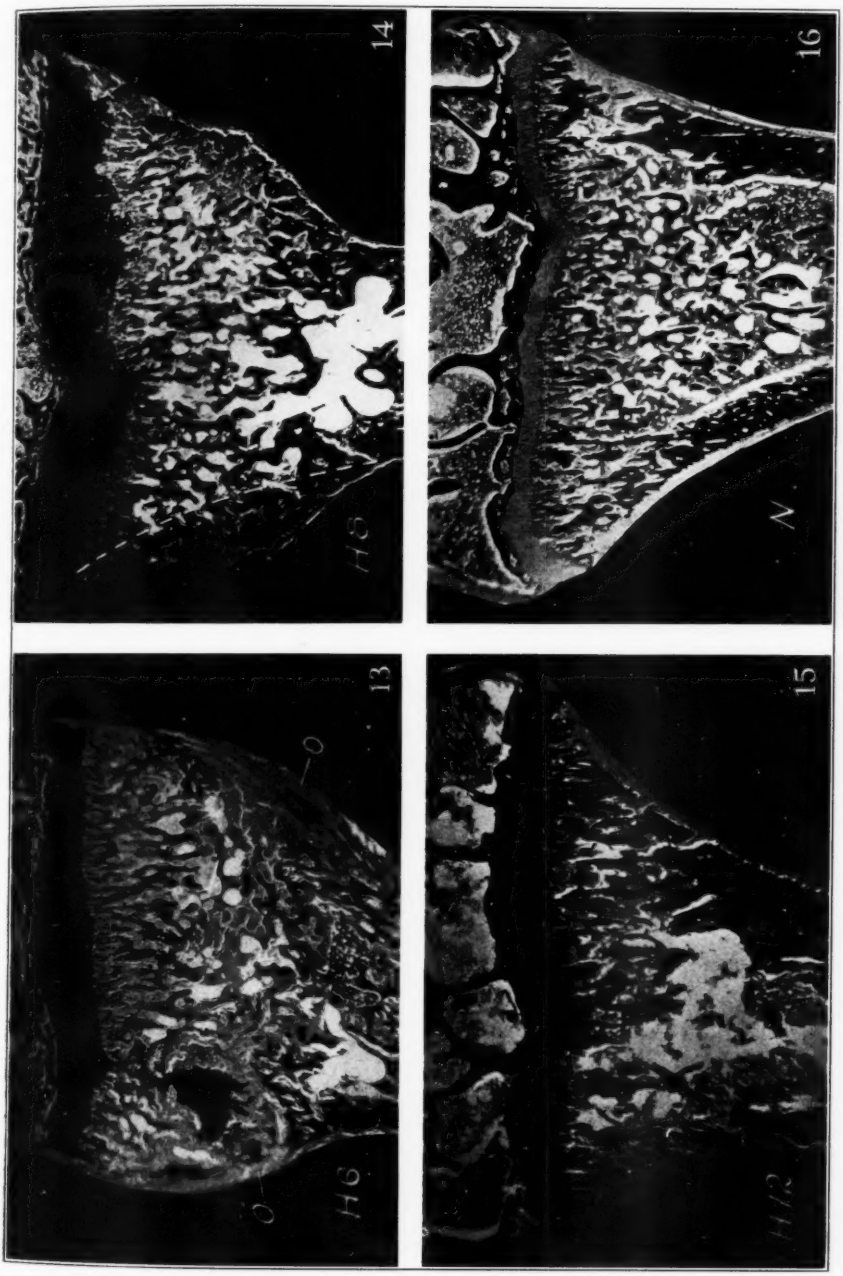

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PLATE 57

FIGS. 17, 18, 19, 20. Series of microphotographs showing healing process in edge of rachitic metaphysis. Sections stained with hematoxylin and eosin. × 100.

FIG. 17. Severe rickets. Shows trabeculae of uncalcified rachitic metaphysis, composed of masses of osteoid enclosing uncalcified cartilage remnants. The marrow in the greatly reduced spaces is almost wholly vascular. At border between cartilage and metaphysis there is considerable accumulation of osteoid, indicating well advanced rickets.

FIG. 18. Healing Stage 2 (*cf.* Figs. 7 and 12). Shows early healing region (Zone 1) about 100 μ wide, where excess osteoid has been removed from the cartilage trabeculae, which have subsequently become calcified. In this zone the marrow spaces have greatly increased in size, in Zones 2 and 3 moderately so. In all zones the marrow has become less vascular and more cellular. Zone 2 shows calcification of osteoid, though not strongly.

FIG. 19. Healing Stage 4. Shows further progress of changes begun in Fig. 18. Zone 1 is much wider than in Stage 2 (400 μ). Calcification shows strongly in cartilage trabeculae of Zone 1 and in osteoid masses of Zone 2.

FIG. 20. Healing Stage 6 (*cf.* Figs. 8 and 13). Zone 1 has become much widened by removal of osteoid and calcification of cartilage trabeculae. Configuration of trabeculae approaching normal, but osteoid layer is still mostly free from calcification.

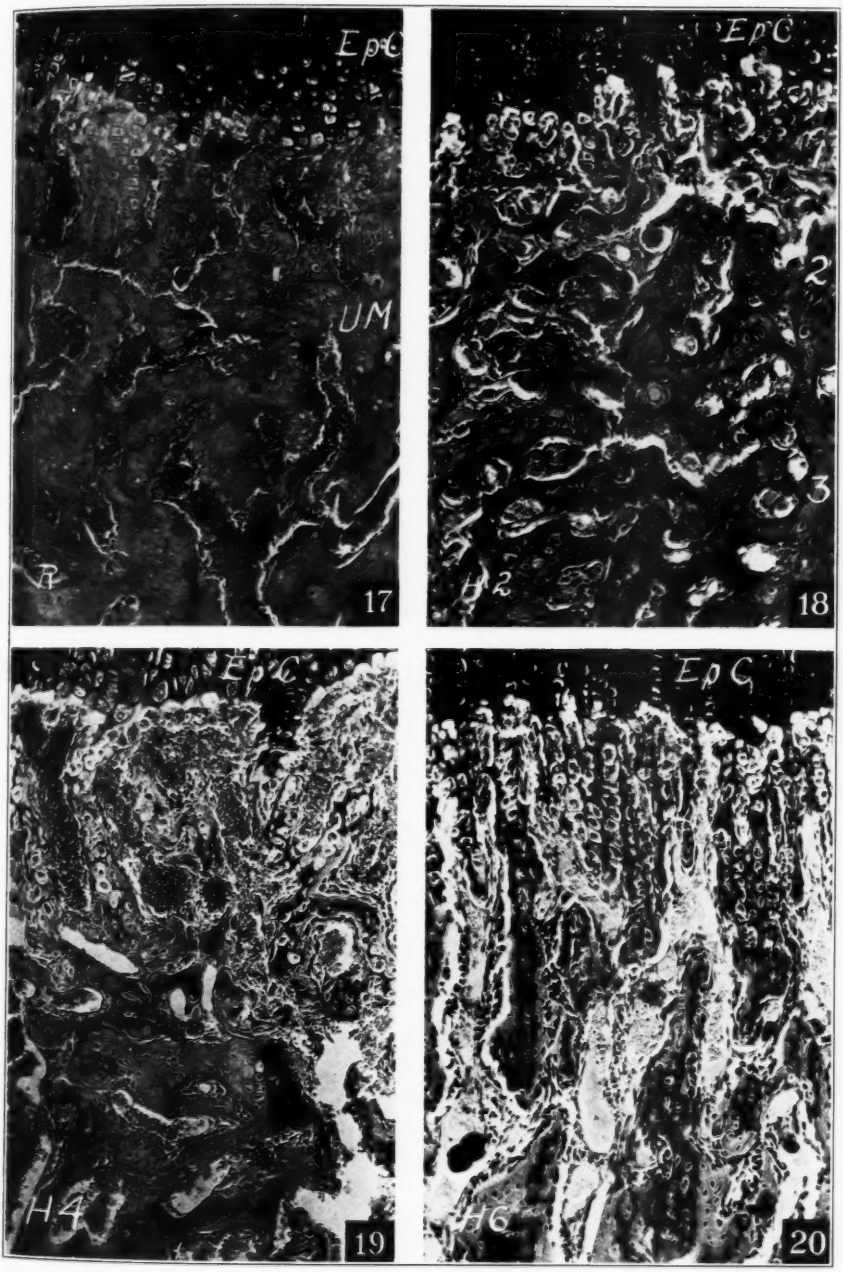

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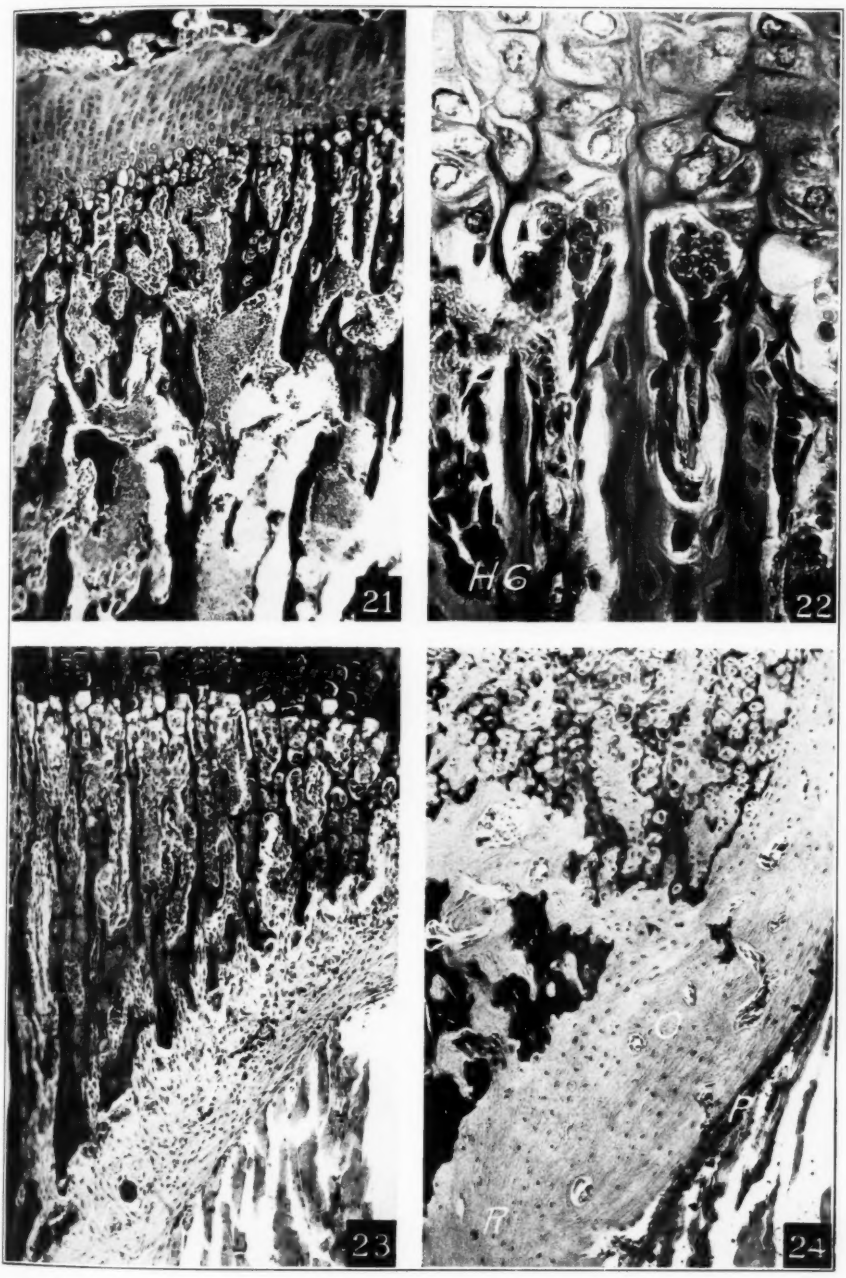
PLATE 58

FIG. 21. Continuation of series on Plate 57. Healing Stage 12. Epiphyseal cartilage has been reduced to normal thickness and has become normally calcified. Calcification and form of bone trabeculae have become normal. Hematogenetic marrow has developed well toward cartilage. × 100.

FIG. 22. Healing Stage 6. Hematoxylin and eosin stain. Chosen to show details of calcification in Zone 1 and edge of epiphyseal cartilage. Note that calcification is in two narrow bands on the two sides of the trabeculae and that it extends into the cartilage only about one lacuna. Osteoid on cartilage trabeculae not calcified at this stage. Note also normal appearance of marrow with well developed osteoblasts. × 450.

FIG. 23. Normal tibia. Hematoxylin and eosin stain. From curved part of bone below epiphyseal cartilage to show mechanism of tubulation process. Note lack of cortical bone, and presence of osteoclasts on ends of trabeculae ending in periosteum. × 100.

FIG. 24. Severe rickets. Region corresponding to Fig. 23. Thick osteoid layer (300 μ) has covered ends of trabeculae, osteoclasts have disappeared; bone removal in this region has ceased, which means that tubulation is not possible (*cf.* Fig. 11). × 100.



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PARS INTERMEDIA BASOPHIL ADENOMA OF THE HYPOPHYSIS *

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Recent reviews of Cushing's pituitary basophilism (Tesseraux,[1] Brauer,[2] Pardee,[3] Bland and Goldstein,[4] and others) indicate that the rôle of the hypophysis in this syndrome is far from being settled. There are certainly many cases of this type in which no basophil adenoma is demonstrable, and some without any hypophyseal tumor of any kind. The 2nd case reported by Ulrich [5] is an example where no evidence of adenoma could be found in any organ. Practically all cases, however, show some hyalinization of the basophils as described by Crooke.[6] Statements to the effect that there is hyperplasia of the basophils of the anterior lobe, but no distinct adenoma, must be taken with considerable reservation because of the failure to recognize the irregular distribution of these cells normally, the lack of quantitative methods (Rasmussen [7]) and insufficient examination of the gland.

Many basophil adenomas have been found without being accompanied by so-called pituitary basophilism (Kraus,[8] Costello,[9] and Susman [10]). Close [11] reported a case where the only symptom common to pituitary basophilism was high blood pressure. The syndrome has occurred in cases purported to show acidophil adenomas of the hypophysis (Reichmann,[12] Konschegg,[13] and Horneck [14]), and where the hypophyseal tumor was strictly chromophobic (Fuller and Russell [15]), or chromophobic with a few scattered basophils (Crile, Turner and McCullagh [16]). The case reported by Bettoni,[17] in which the anterior lobe was almost completely occupied by a chromophobic adenoma, probably belongs in this general category. In a number of instances it has been impossible to be definite as to the basophilic character of the tumor. In the 1st case reported by Ulrich,[18] slight indications of the basophilic character of the granules could be obtained only with great effort and then only in a few of the larger cells. In all recent extensive lists of cases there are several in which the type of adenoma was not clearly established.

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A high percentage of cases with the syndrome of pituitary basophilism have either an adrenal cortical tumor or distinct hypertrophy of the adrenal cortex. McQuarrie, Johnson and Ziegler [19] have emphasized the fact that the electrolyte pattern of the plasma in most respects may be diametrically opposite to that found in Addison's disease, and hence suggest "hypercorticoadrenalism" as the most expressive name of the syndrome. This is further strengthened by the studies of Jores,[20] and by Anderson and Haymaker,[21] who found evidences of increased adrenal cortical hormone in cases of Cushing's disease. Bauer [22] is rather emphatic that the syndrome is a form of interrenalism, while Kraus [8] argues that both the hypophyseal and adrenal changes are compensatory in nature, secondary to fat metabolism and associated metabolic disturbances. Various differential diagnoses between adrenal cortical hyperfunction, basophil adenoma of the hypophysis and arrhenoblastoma of the ovary have been attempted (Goldzieher and Koster [23]), but great difficulty still exists as is shown by a case (Norris [24]) presenting a malignant ovarian tumor. The report by Walters, Wilder, and Kepler [25] on the suprarenal cortical syndrome represents the situation well. A very recent case (Pons and Pappenheimer [26]) with the "classic" features of so-called pituitary basophilism showed neither a basophil adenoma nor hyalinization of the basophil cells and was finally designated "renal hyperparathyroidism."

It is, therefore, advisable to record all basophil adenomas of the hypophysis so that their significance may be better understood. Apparently it is necessary to allow for a fair percentage of chance coincidence. The cases reported here are of special interest because the adenomas appear to have arisen from the same source as the basophilic cells that normally invade the neural lobe. Since these invading basophils normally differentiate from the pars intermedia (Rasmussen,[27] Roussy and Mosinger,[28] Plaut,[29] and Lennon [30]) we are apparently dealing with pars intermedia tumors. The migration of basophilic cells into the neural lobe has been greatly discussed in recent years, particularly in connection with hypertension (Cushing [31, 32]). Ahlström,[33] Gómez Marcano,[34] and Leary and Zimmerman [35] present additional data that have been interpreted as supporting more than mere chance correlation between the invasion and hypertension. Yet Gómez Marcano does

not consider that a constant relation exists between the degree of basophilic invasion and the height of the blood pressure. Most observations, however, do not favor any relation (Kraus,[36] Spark,[37] Biggart,[38] Berblinger,[39] Butt and Van Wart,[40] Seecof,[40] Boyd,[40] Hawking,[41] Scriba,[42] Plaut,[29] Parsons,[43] and Rasmussen [44]), although a few of these authors think there is some connection between the abundance of basophils in the *anterior* lobe and high blood pressure. Again, Kraus thinks this basophilic invasion may be related more directly to adiposity than to blood pressure, particularly in eclampsia.

In connection with diffuse basophilia of the anterior lobe and its relation to blood pressure, it should be noted that only in the reports by Hawking [41] and Rasmussen [44] were the cells enumerated, and both these studies show that the increase in percentage of basophils in essential hypertension is not regular enough to be regarded as possessing etiological significance.

The following cases are presented as additional evidence that great caution is still necessary in estimating the effects of basophil adenomas of the hypophysis.

Case Reports *

Case 1: The tumor was found unexpectedly at autopsy in a well developed 77 year old white male who was admitted to the University of Minnesota Hospital May 10, 1937 because of nocturia, urgency, incontinence and loss of weight of 9 months duration, although he had had mild urinary symptoms for many years. Slowness of speech and comprehension had been evident for a year. Occasional edema of the ankles occurred. The blood pressure 1 year before admission was 260/90 mm. Hg. He had slight dyspnea but no other cardiorespiratory symptoms.

On admission the systolic blood pressure ranged from 200–260 mm. Hg., and the diastolic from 115–150 mm. Hg. An occasional extra systole was noted. Pitting edema of the ankles was present. The prostate was moderately enlarged. Residual urine amounted to 750 cc. and contained traces of albumin and numerous blood cells but no sugar. The blood showed 85 per cent hemoglobin, 10,300 leukocytes and 62 per cent neutrophils. The blood urea nitrogen was 22 mg.; phenolsulphonephthalein 44 per cent; Wassermann negative.

Within 2 days there developed thickness of speech, dropping of left side of mouth, deviation of the uvula and tongue, modified reflexes and

* We are indebted to the Department of Surgery, University of Minnesota, for permission to publish these cases.

weakness of the left extremities. The electrocardiogram indicated auricular fibrillation and ventricular extrasystoles. A cystostomy was done a week later and the prostatic condition greatly improved. Symptoms of cerebral thrombosis persisted and the patient died on the 18th day.

Autopsy Findings

The autopsy was performed 45 minutes after death. The body was emaciated, 171 cm. in length and weighed about 115 pounds. A subcutaneous lipoma 4 cm. in diameter was present in the left hypochondriac region. The heart weighed 430 gm. and showed slight left ventricular hypertophy but no fibrosis of the myocardium. The mitral and aortic valves, coronary arteries and the root of the aorta showed moderate senile atheromatosis. The kidneys were quite smooth and weighed 130 and 150 gm. respectively. The parenchyma was pale and slightly fibrous. Microscopic sections revealed slight senile arteriosclerotic changes but no hypertensive changes. Moderate bilateral hydronephrosis and hydroureter were present. The bladder was markedly trabeculated. There was moderate hemorrhagic cystitis. The lateral lobes of the prostate were slightly hypertrophied, there was a fibrous median bar formation, and sections showed irregular nodular adenocarcinomas. The adrenals were normal. The left testis was markedly atrophic. The thyroid was small, brown and fibrous with an adenoma (3 by 3.5 by 4.5 cm.) at the left lower pole. This adenoma contained a cyst measuring 2 cm. in diameter with a calcified wall. The parathyroids were normal. No thymic tissue could be seen.

The blood vessels at the base of the brain were sclerotic and greatly reduced in size. After injection with formalin the brain was found on section to contain numerous small cystic areas in the region of the right internal capsule and a few in the left side. On the right was a fairly large area of softening involving the internal capsule and basal nuclei. Microscopic examination revealed no cellular reaction around these cavities. Some were of recent origin.

The upper aspect of the sella turcica appeared to be normal but dissection uncovered a tumor about 1.5 cm. in diameter adherent to the right side of the hypophysis and attached to its lower pole. The adenoma extended underneath the right internal carotid

artery (Fig. 1), and depressed the roof over the right side of the sphenoid sinus, but did not appear to have invaded the bone at any point. Discovery of this neoplasm stimulated a more careful examination of the body for further evidences of endocrine disorders, but the body build, muscular development and hair distribution were all normal. There was no evidence or history of obesity, purple striations or acromegaly. After serial sections of the hypophysis showed the tumor to be a basophil adenoma, the records were carefully checked but no signs or symptoms indicative of pituitary basophilism existed, except the high blood pressure.

The hypophysis was somewhat asymmetrical on account of the pressure of the tumor on the right side. The tumor was cut so that part of it remained attached to the hypophysis and the rest was removed with the internal carotid artery. Both blocks of tissue were fixed in formalin, cut at 5 μ and stained with the Mallory-Heidenhain and hematoxylin-eosin stains. A rather sharp boundary line, which gradually merged into the connective tissue capsule of the anterior lobe more anteriorly, separated the new-growth from the anterior lobe (Fig. 2). As the series of sections were followed toward the lower pole the tumor became denser, large nodules of strongly basophilic cells appeared (Fig. 3) and there was continuity with a great mass of similar cells that infiltrated the neural lobe (Fig. 3).

A careful study of serial sections indicates that the outgrowth has been from the lower pole of pars intermedia — a region where basophilic invasion of the neural lobe is prevalent. The cells in well preserved portions of the adenoma are identical with the cells not only in the adjacent neural lobe but also in remote regions of this lobe (Fig. 2) where they have unquestionably been derived in the usual manner from the pars intermedia. The cells are variable in size. None are excessively large. They are distinctly granular but only occasionally vacuolated. A high power view of the area marked + in Figure 3 is shown in Figure 5. Much of the tumor contains only small, irregular basophilic islands (the dark areas in Figure 4) in which the cells are degenerating and contain only a few basophilic granules. The intervening region consists of a loose network of connective tissue invaded by neutrophils and containing some scattered indifferent cells with little or no cyto-

plasm and cells in various stages of degeneration. In limited areas there is diffuse hemorrhage and fibrin formation. As a whole the tumor is poorly vascularized. There is a small indifferent staining region in the anterior lobe (Figure 2, just below the end of the leader to the anterior lobe) which appears to be degenerative. No hyalinization of the basophils is present.

Comment: Owing to the advanced age of this individual, one would hesitate to associate the high blood pressure with the basophilia. The probability of mere chance coincidence would appear to be too great. It should also be recalled that hypertension is not uncommon in acromegalia which is due to acidophil adenoma. Jéquier [45] found this to be true in over a third of the cases. Hypertension has been found in 60 per cent of acromegalic females over 40 years of age. The literature is well reviewed by Houssay.[46]

CASE 2: A white female, 55 years of age, was admitted to the University of Minnesota Hospital Nov. 22, 1937, with a strangulated umbilical hernia. Because of the condition of the patient no physical findings, except those immediately connected with the hernia, were obtained. She was operated upon immediately. Some dark loops of intestine near the hernial sac became progressively better in color, showed active peristalsis, and were, therefore, returned to the abdomen and the hernia repaired. The systolic pressure was down to 70 mm. Hg. Transfusion of 700 cc. of blood and 800 cc. of normal saline brought the systolic pressure to 90 mm. Hg. where it stayed. The patient was cyanotic and was put in an oxygen tent but expired the same day.

Slightly less than a year preceding death the patient was examined by a local physician because of dyspnea and a fainting spell. Her weight at that time was 225 pounds and the blood pressure was 204/96. The lower parts of the legs were edematous. The urine was free from sugar and albumin. Six months previously she had been examined by another physician during an acute attack of influenza. The hypertension and dyspnea were evident at that time. The heart sounds were slightly irregular and rather weak. A moderate number of granular casts were present in the urine.

Obesity and hair on the face appeared shortly after the birth of her first child, 33 years ago. She had three normal children subsequently and continued to menstruate regularly till 51 years of age. She even menstruated once when 54 years of age.

Autopsy Findings

The autopsy was performed about an hour after death. The body was very obese, 168 cm. in length and about 210 pounds in

weight. The obesity was marked on the trunk and upper portions of the extremities. There was slight edema of the legs and numerous varicose veins. Papillomas up to 1 cm. in diameter were present on the right labium majus, the inside of the right thigh, the right nipple, and small ones were present on the upper eyelids. A heavy growth of thick stiff hairs was present around the mouth and on the chin. Purple striae were clearly evident on the abdomen. The outward features were characteristic of Cushing's pituitary basophilism so that the body was carefully examined for further evidences of this syndrome.

The umbilical hernia had been repaired and was in good condition and there was no peritonitis or ascites, but about 6 feet from the ileocecal valve was a segment of small intestine 10 cm. long which showed early gangrene. The abdominal subcutaneous fat was 6 cm. in thickness. The heart weighed 575 gm. There was marked ventricular hypertrophy on the left and slight hypertrophy on the right. The myocardium of the anterior portion of the left ventricle showed a moderate degree of old spotty fibrosis but no large infarction. The valves were normal. The foramen ovale was closed. Atherosclerosis was moderate in the coronary arteries and much of the aorta. There was a stone 7 mm. in diameter in the common bile duct and the gall bladder was tightly filled with about fifty stones. A moderate degree of fat replacement was evident in the pancreas. The kidneys were essentially normal on gross appearance but sections showed a moderate degree of hypertensive changes in the small arteries and arterioles. The adrenals were normal. The uterus weighed 225 gm. and contained about a dozen interstitial myomas up to 2 cm. in diameter. The cervix uteri was eroded and cystic. The uterine tubes and right ovary were normal. The left ovary was replaced by a multilocular cystic mass 7 by 8 by 10 cm. which contained clear yellow fluid. The wall of one of these cysts had a few early papillomatous growths. There were a few small cysts around the right ovary. At the lower pole of each lobe of the thyroid was an adenoma of the mixed type. The one on the left was 3 cm. in diameter and was calcified, while the one on the right was soft, pale in color, and measured 4 cm. in diameter. Sections of the thyroid indicated moderate atrophy and fibrosis with lymphocytic foci. The parathyroids were normal. No thymic tissue was visible.

The brain weighed 1210 gm. and showed nothing unusual beyond a moderate degree of sclerosis in the vessels at the base. The sella turcica was normal. The hypophysis weighed 0.682 gm. and except for a moderate degree of cupping on top gave no outward indications of any abnormality. It was fixed in formalin, sectioned serially and stained as in Case 1. A definite basophil adenoma about 3 mm. in diameter was found on the left side of the upper region of the pars intermedia and extending into the rim formed by the depression of the cerebral part of the upper surface of the gland (Figs. 7).

On microscopic examination the cells of the adenoma are found to be strongly basophilic, densely packed (Fig. 6), and to compress the upper left pole of the anterior lobe from which the adenoma is separated by a sharp boundary line in which are flattened colloid vesicles and other traces of pars intermedia (Figs. 7 and 8). At about the middle of the series it becomes less compact and merges into a stratum of basophilic cells which extends across the entire anterior portion of the neural lobe and enlarges at the right margin so that there is a more or less symmetrical bilobed basophilic area throughout the rest of the gland to the lower pole (Fig. 9). The cells of the adenoma proper (Fig. 6) present the structural features of the basophilic cells that normally migrate into the neural lobe. In the interior is an irregular fluid-filled space (Fig. 7) which has no definite wall. There are no indications of degeneration.

Numerous, small circumscribed masses of basophils (Fig. 10) are found throughout most of the anterior lobe, particularly in the upper part of the gland and near the pars intermedia. Larger areas of more diffuse basophilia are intermingled with the above so that there is an excess of basophilic cells in the anterior lobe also. An actual differential count of the cells of the anterior lobe showed that there were 39 per cent chromophobes, 30 per cent acidophils and 31 per cent basophils. The average percentage of basophils in females normally is about 7 per cent and they rarely reach 17 per cent.[7] There is no hyaline change such as is almost always evident in pituitary basophilism. This hyalinization is regarded by Crooke as an expression of altered physiological activity, while Severinghaus [47] thinks it is a degenerating process.

Comment: In spite of the adenoma and the excess number of

basophils in both lobes of the hypophysis and the presence of several of the characteristics of Cushing's syndrome, there were no menstrual disturbances, glycosuria, osteoporosis, hypertrophy of the adrenal cortex, or hyaline change in the basophilic cells of the hypophysis. The obesity had existed for nearly 33 years. Crooke and Russell would exclude this from the list of true pituitary basophilism.

At least 2 other basophil adenomas apparently originating from the pars intermedia are on record — the Raab-Kraus case and Case 6 (fatal eclampsia) reported by Cushing — both described in detail by Cushing.[32] They were wholly within the posterior lobe. A 3rd case, published by MacCallum, Futcher, Duff and Ellsworth,[48] is undoubtedly an anterior lobe tumor. It was strictly within the anterior lobe but posteriorly extended to the intermediate region. Their principal reason for considering it of pars intermedia origin was the mistaken idea that the copper hematoxylin method stains the basophils of the anterior lobe dark blue. The tumor by ordinary stains was basophilic but did not stain with copper hematoxylin. The facts are that it is the acidophils of the anterior lobe that stain deep blue with copper hematoxylin,[49] and neither the basophils of the anterior lobe nor those that arise from the pars intermedia take this stain strongly.

Summary

Two cases of basophil adenoma originating from the pars intermedia are described. In 1 case the only symptom at all referable to the hypophysis was high blood pressure. On account of the old age of this individual (77 years) the association of the adenoma with the hypertension is questionable. In the 2nd case a number of the major characteristics of pituitary basophilism (adiposity, striae atrophicae, hirsuties, high blood pressure, florid face) were present. In neither case were there any hyaline changes in the basophils.

There was considerable diffuse invasion of the neural lobe by basophils in both cases. The great bulk of data, however, does not indicate that there is any direct significant correlation between this invasion and hypertension.

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DESCRIPTION OF PLATES

PLATE 59

FIG. 1. Case 1. Drawing of the hypophysis and adenoma in relation to the internal carotid arteries. Viewed from above. The lateral margin of the tumor underneath the right carotid artery is indicated by a broken line.

FIG. 2. Case 1. Microphotograph of a horizontal section through the hypophysis and part of the adenoma, which is closely applied to the right side of the hypophysis but sharply separated from the latter at this level. A large number of basophilic cells have invaded the neural lobe quite remote from the tumor. A small, circumscribed degenerating area is seen at the end of the leader in the anterior lobe.

FIG. 3. Case 1. Microphotograph of a horizontal section through the lower pole of the hypophysis at about the middle of the adenoma which is continuous with the basophils of the pars intermedia, which completely encircle the neural lobe at this level. A conspicuous nodule of strongly basophilic cells in the adenoma is indicated by a white +. Except for such nodules the tumor is for the most part loose like the lower third of the figure. At slightly lower levels, where there is no longer any evidence of the neural lobe, the continuity between the adenoma and the pars intermedia is even more evident, but an illustration from this lower level would be less informative than the one here reproduced.

FIG. 4. Case 1. Microphotograph at a higher magnification showing that portion of the tumor which is in contact with the internal carotid artery. The wall of the artery is shown in the lower part of the figure. The irregular scattered dark areas are mostly degenerating basophils.

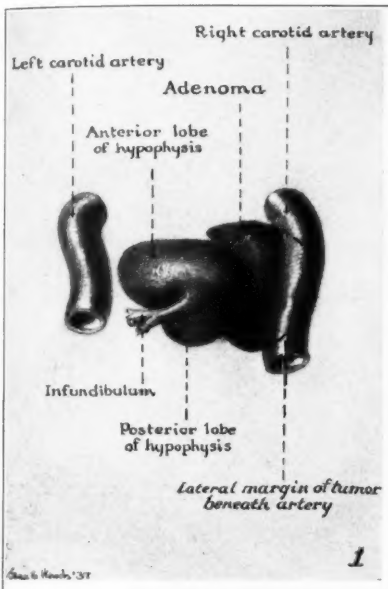

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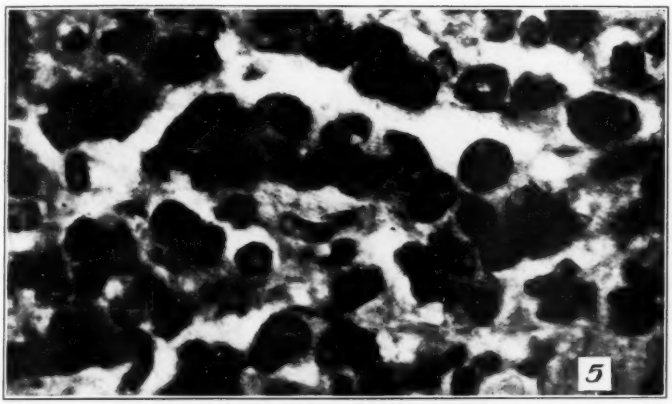
FIG. 5. Case 1. High power view of the area in Fig. 3 marked with a white +. The cells are variable in size and in degree of granulation and only occasionally vacuolated. In structure and staining reaction these cells are indistinguishable from the basophils that normally differentiate from pars intermedia and more or less invade the neural lobe.

FIG. 6. Case 2. Microphotograph of the center of the basophil adenoma shown in Fig. 7. The cells are small, closely packed and moderately rich in granules. Palisading around the blood vessels is rarely evident. There are no indications of active degeneration.



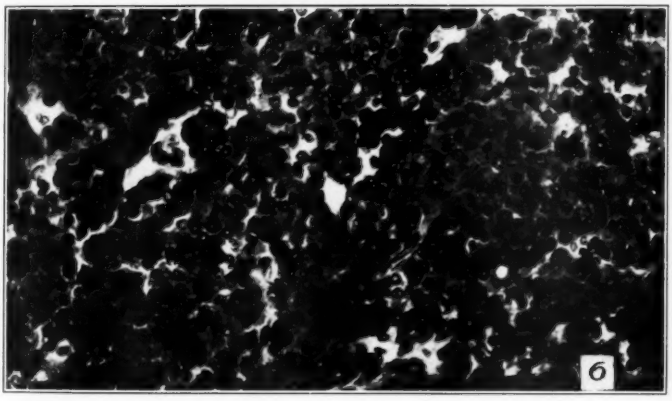


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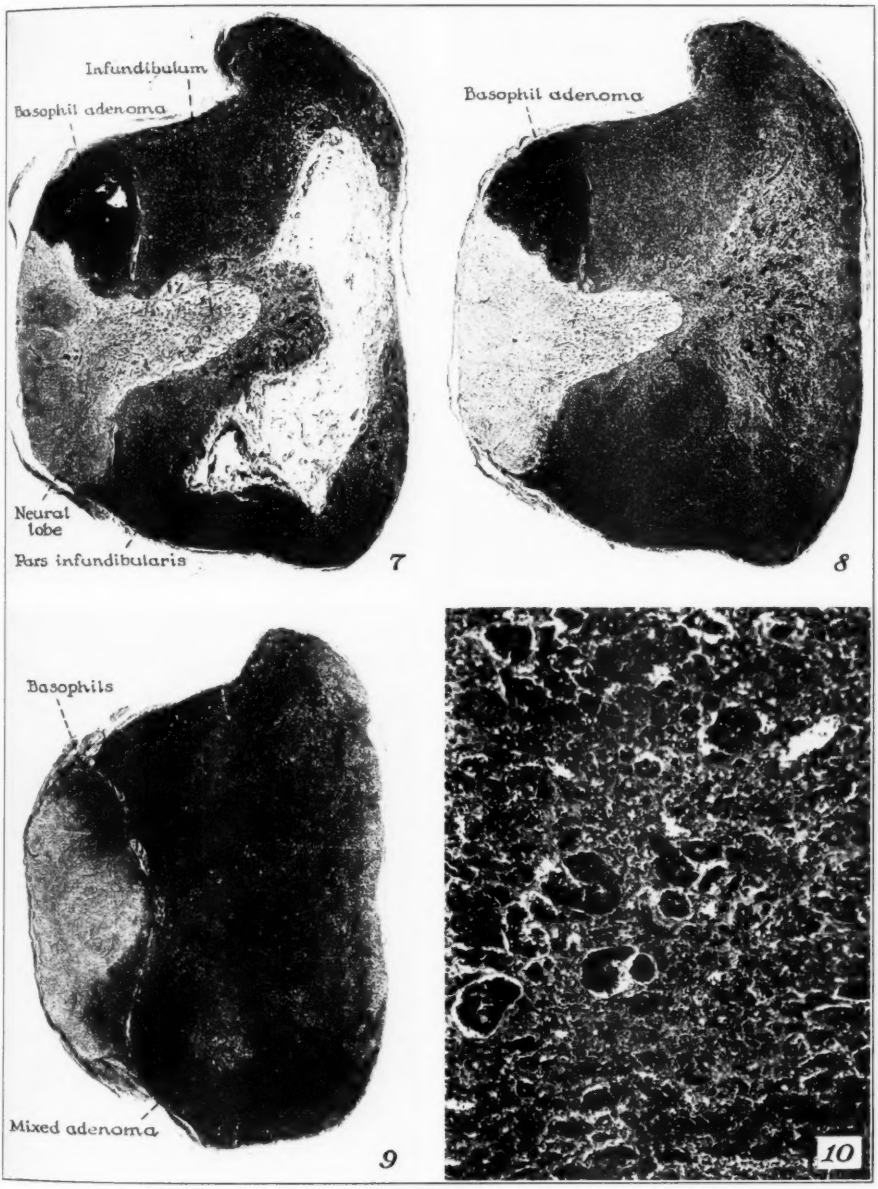
FIG. 7. Case 2. Microphotograph of a horizontal section through the upper rim of the hypophysis showing the location and general dimensions of the basophil adenoma. Part of the residual lumen and other evidences of the boundary between the anterior lobe and the pars intermedia are still present and indicate that the tumor is strictly within the posterior lobe.

FIG. 8. Case 2. Microphotograph of a horizontal section about a millimeter lower than Fig. 7 showing the adenoma in the same relative position.

FIG. 9. Case 2. Microphotograph of a horizontal section through the middle of the hypophysis and entirely below the adenoma where it has been replaced by a looser mass of basophilic cells derived from the pars intermedia, into which the tumor gradually merges. Diffuse basophilic invasion is present at this and all lower levels on the other side as well and to a lesser extent in the intervening region. In the anterior lobe is a small area having the appearance of a mixed adenoma containing many weakly basophilic cells.

FIG. 10. Case 2. Microphotograph of a typical area of the anterior lobe of the hypophysis showing numerous small circumscribed masses of deeply basophilic cells. Normally such groups of cells are not rare but there is an excessive number in this specimen.



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A STUDY OF THE SUBMAXILLARY GLAND VIRUS OF THE GUINEA PIG *

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INTRODUCTION

Intranuclear inclusions have been described in the salivary glands of the human infant and stillborn fetus,[1] in the monkey (*Cebus fatuellus*),[2] guinea pig,[3] rat,[4] mouse,[5] hamster,[5] and mole.[6] The presence of a filterable virus in association with these inclusions has been demonstrated only in the rodents. The benign character of these infections, and the strict host specificity and organ selectivity of these viruses set them apart as a group of particular interest. The first of the submaxillary gland viruses to be discovered was described by Cole and Kuttner [7] in the guinea pig in 1926, and since that date the results of a number of experimental studies of this virus have appeared in the literature. The present report deals with the submaxillary virus of guinea pigs with respect to spontaneous infections, the morphology and nature of the inclusion bodies, infectivity and virulence of the virus, and the immunological phenomena associated with infection.

METHODS AND MATERIALS

Virus emulsions for experimental infections were prepared from the submaxillary glands of guinea pigs supplied by local dealers, or from artificially infected stock animals. The salivary glands were removed under ether anesthesia from 3 or more animals and ground in a mortar with sterile sand. The pulp was taken up in approximately 1 cc. of physiological saline per gland. Gross particles were allowed to settle out or were thrown down by light centrifugation. The supernatant fluid was considered as a stock emulsion for purposes of dilution. Intracerebral injections of young guinea pigs and fetuses of the same species were made in 0.05 to 0.1 cc. doses. Fetal inoculations were made by the tech-

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nique described by Woolpert,[8] employing pregnant guinea pigs in the 4th and 5th weeks of gestation. Unless otherwise specified, all tissues examined histologically were fixed in Zenker's solution, embedded in paraffin and stained with eosin-methylene blue or eosin-azure.

Spontaneous Infections

The cellular changes associated with the submaxillary gland virus were first observed by Jackson in 1920.[3] The inclusions are found in the epithelial cells of the salivary ducts and less often in the renal epithelium. In the submaxillary gland the affected cells undergo a marked hypertrophy, a reaction that is characteristic of many virus infections and to which Unna applied the term "ballooning degeneration." Scott and Pruett,[9] who made a detailed study of the volumetric changes in the cell, found that under the influence of the virus the cytoplasm may increase as much as 300 per cent and the nucleus as much as 400 per cent in volume.

Lesions may occur in the mucous accessory portion of the submaxillary gland, but are most often found in the serous part where they are commonly distributed in the smaller ducts near the periphery of the lobules. A single section may disclose one or more infected ducts with or without lymphocytic infiltrations varying from a cuff three or four cells deep to massive infiltrations which completely obscure the architecture of the duct and surrounding acini. Inclusion laden cells in the latter type of lesion may stain very poorly and be difficult to find. Such reactions are observed only in old adult guinea pigs and possibly indicate a slow resolution and elimination of infected foci.

In the kidney (Fig. 5), where the epithelium of the convoluted tubules becomes infected, the affected cells do not undergo the marked hypertrophy seen in the salivary gland. The intranuclear inclusions resemble those observed in the submaxillary gland and rarely acidophilic bodies are found in the cytoplasm of affected renal epithelium.

The incidence of spontaneous infection with the submaxillary gland virus in stock animals varies widely. Andrewes [10] in England reported 32 per cent. In this country Jackson found inclusions in the glands of 54 per cent, and Cole and Kuttner in 84 per cent of the animals they examined. The incidence we have found in the

stocks of local dealers ranged from 7 to 74 per cent. Casual observation suggests that there is a relation between the sanitary conditions under which the animals are kept and the frequency with which positive glands are encountered. Histological evidence of infection in the glands of guinea pigs less than 1 month old is seldom found. However, that general absence of infection in young animals is not due to temporary resistance is shown by the fact that such animals are susceptible to intracerebral [11] and other routes of inoculation. Spontaneous infection may take place in the first few days of life, but 10 to 15 days must elapse before typical lesions can readily be demonstrated in the glands. Of 12 14 day-old guinea pigs examined, we found microscopic evidence of infection in 1. Cole and Kuttner found typical lesions in the glands of 3 of 43 guinea pigs, most of which were less than 1 month old.

Characteristic inclusions were present in the renal epithelium of 5 of 62 of our stock animals (8 per cent). The only report in the literature mentioning the incidence of spontaneous lesions in the kidney is that of Jackson, who reported the presence of inclusions in the renal epithelium of 12 of 44 adults (27 per cent).

The natural method of transmission of the virus has not been determined under experimental conditions. The presence of inclusions in organs such as the salivary glands and kidneys suggests that their secretions may be infectious. Using Berkefeld N filtrates of urine and saliva from known infected animals, we were unable to infect young guinea pigs by intracerebral inoculation. Two weeks after injection histological examination of the brain, salivary glands and kidneys revealed no characteristic lesions.

Morphology and Nature of the Inclusions

Preliminary attempts to study the structure of the intranuclear inclusions in frozen sections of the salivary glands were abandoned because of the fragile nature of the tissues and the scarcity of isolated cells suitable for observation. It was found that the meningeal exudate of intracerebrally inoculated animals offered greater possibilities for the study of inclusions in fresh unfixed cells. Preparations for examination were made by clipping small fragments of infected meninges from the ventral surface of the brain and placing them in a small drop of serum or saline on a

slide previously coated with a solution of Janus green and neutral red. It was observed that mononuclear cells containing inclusions take up extremely small amounts of the stains as compared with uninfected cells. The former cells are further characterized by their great size and distended nuclei (Fig. 1). Lying within the nuclear membrane and sometimes attached to it by barely perceptible filaments, the inclusion appears as a closely packed mass of refractile granules. As a rule only one such mass occupies the nucleus and the surrounding nucleoplasm is optically empty. As in the fixed and stained preparations, small clumps of chromatin can be seen in apposition to the nuclear membrane.

The granular character of the inclusion as seen in fresh mounts suggested that appropriate methods might disclose corpuscles of the sort found in certain other virus inclusions. Sections 5 to 7 μ thick were prepared from Zenker-fixed submaxillary glands of spontaneously infected guinea pigs. They were treated in the usual manner with xylol, alcohol, iodine and sodium thiosulphate, but were examined in the unstained condition in glycerin mounts. In such preparations the corpuscular nature of the inclusion is strikingly apparent on direct examination, though very difficult to photograph because of the absence of contrasting stains and the oblique lighting required for their visualization (Fig. 2). They appear as very small, round or oval bodies closely packed together and embedded in a less refractile matrix or ground substance. Because of the type of illumination employed it was not possible to measure them accurately.

Various dyes were used in attempts to stain the component parts of the inclusion. The best results were obtained by overstaining with Harris' hematoxylin and differentiating in an approximately half saturated solution of picric acid. Stained by this method the corpuscles appear discrete but considerably smaller than those seen in the unstained and undehydrated glycerin mounts (Fig. 3). In a few of the inclusions so stained these minute bodies are arranged in more or less concentric rings and resemble the elementary bodies reported in infectious ectromelia inclusions photographed by darkfield illumination and ultraviolet light.[12] On the basis of the evidence presented we propose that the nuclear inclusion is composed of units comparable to the elementary bodies found in some other virus infections.

Stains for fat, fibrin and amyloid substance showed that these substances are not present in the inclusions in amounts detectable by the usual methods. Silver impregnation methods added nothing noteworthy to our information on the structure of the inclusion.

We were able to examine tissues prepared by a modification of the Altmann technique.[13] * This method involves fixation by rapid freezing in liquid air and desiccation *in vacuo*. The dry tissues are then quickly infiltrated and embedded in paraffin and sectioned in the usual manner. The method has the great advantage of yielding tissues that are not denatured by chemical fixatives and are therefore especially suitable for microchemical studies. Sections of infected submaxillary glands were thus prepared and stained with Feulgen's reagent for thymonucleic acid. Both the cytoplasmic and intranuclear inclusions react positively but with different intensity. The cytoplasmic bodies stain rather solidly in contrast with the intranuclear inclusions which often present a marked honeycombed appearance. This seems to be due to the affinity of the matrix material alone for the staining reagent. The elementary bodies retain none of the stain, thus giving the inclusion a vacuolated appearance (Fig. 4).

Other microchemical tests were made on sections prepared by the Altmann technique. Solubility tests (test interval of 5 minutes in each instance) were made with the following reagents: petroleum ether, xylol, ethyl alcohol, physiological saline solution, 0.5 per cent acetic acid, and normal HCl at room temperature and at 60° C. None of these reagents dissolved or materially altered the appearance of the inclusions. Pepsin and HCl digested the connective tissue but not the inclusions. A 2.5 per cent solution of Na_2CO_3 removed all chromophilic matter from the inclusions and all nuclei. The Millon test for protein was positive. While none of these microchemical tests has a high degree of specificity, collectively they indicate the protein nature of the matrix-substance of the inclusion bodies.

Infectivity and Virulence

Experimental transmission of the virus is readily effected in young guinea pigs by subcutaneous, intraperitoneal, or intrave-

* Through the kindness of Dr. I. Gersh, then of the Department of Anatomy, University of Chicago.

nous injections of infective gland emulsions. After 10 to 15 days, inclusions are demonstrable in the salivary glands. As in spontaneous infections, the introduction of the virus by any of these routes is not followed by any marked symptoms of disease. However, when young uninfected guinea pigs are inoculated intracerebrally, they succumb to a specific meningitis. The time of survival or death of the animal and the incubation vary with the potency of the inoculum. In those animals that die acutely the incubation period is usually 5 to 8 days. The symptoms include ruffling of the haircoat, fever, tremors, relaxation of the urethral and anal sphincters, circus movements, and finally prostration. Death is generally preceded by a marked fall in temperature.

Histological examination of the brains of guinea pigs that have succumbed to intracerebral inoculation usually reveals a marked mononuclear meningitis, characterized by the presence of large numbers of intranuclear inclusions in the cells of the exudate. When the incubation lengthens to 15 to 18 days the symptoms are less marked, and histologically the lesions are more circumscribed and the inclusions are scant.

The effect of dilution on the infectivity of the virus is shown by titration in young guinea pigs. The results of such a titration are shown in Table I.

All of the animals that received the undiluted and the 1:10 dilution of the virus emulsion died. Sections of the brains of these 4 guinea pigs showed a typical meningitis with intranuclear inclusions in the exudate. The 2 that received the 1:100 dilution responded with a definite temperature rise and minor nervous symptoms, but both survived. The 3 animals that received the 1:1000 dilution manifested no significant symptoms of infection, and histological examination of the salivary glands of 2 of these animals after 7 weeks revealed no evidence of infection. Gland emulsions, when diluted 1:100 and 1:1000 produced fatal infections in guinea pig fetuses, whereas similar dilutions of the virus produced no more than a febrile reaction in 2 weeks old guinea pigs. The fetus, therefore, proves to be a more delicate test animal for the presence of the virus. This fact is doubtless due to the generalized character of the infection in the fetus. In it lesions containing typical inclusions develop in the meninges, liver, placenta and other organs, in contrast with the localized inflammatory response in the meninges of young postnatal animals.

TABLE I

Titration of Submaxillary Gland Virus Emulsion in Young Guinea Pigs

Animal No.	Inoculum		Days after inoculation							Result
	Dilution	Dose	1	2	3	4	5	6	7	
1	Undiluted	0.10	−	+	+	+	++	++		D 7
2	Undiluted	0.05	+	+++	+++	++	−			D 6
3	1:10	0.10	−	++++	+++	+++	+++			D 5
4	1:10	0.05	−	+++	+++	+++	++	++	+	D 11
5	1:100	0.05	−	++++	+++	+++	+++	++++	+++	S
6	1:100	0.05	−	+	++	+	+++	++	+++	S
7	1:1000	0.10	−	−	−	−	−	−	−	S
8	1:1000	0.05	−	−	−	+	+	+	+	S
9	1:1000	0.05	−	−	−	+	−	+	+	S

− = 39; + = 39.0–39.5; ++ = 39.6–40.0; +++ = 40.1–40.5; ++++ = 40.6–41.0 degrees C.
D = day of death
S = survival

Immunity

Although no detailed study of the immunological phenomena associated with the submaxillary gland virus has been made in the present investigation, certain facts and observations have been brought to light which have a bearing on this question.

Cole and Kuttner reported that spontaneously infected animals were refractory to intracerebral inoculation, but Kuttner [14] was later unable to satisfy herself as to the presence of neutralizing antibodies in the serum of infected adults. Andrewes [10] subsequently showed that if the serum from immune animals was present in the tissue culture medium, inclusion bodies were not formed in susceptible cells.

Although the guinea pig placenta is of the hemochorial type (Grosser's classification) and thus is well suited to the passage of maternal antibody to the fetus, our fetal inoculation experiments have given no evidence that there is any protection conferred upon the fetuses of an immune mother. Likewise, the young of non-immune and immune mothers have been found equally susceptible to the virus. No increased resistance to infection occurred in newborn guinea pigs which were allowed to suckle immune mothers.[15] It therefore appears that passive immunization *in utero* or *postpartum* is not an important factor in resistance.

Certain observations in the fetal inoculation experiments suggest that the duration of active immunity in the spontaneously infected adult is dependent on the existence of more or less active lesions in the salivary glands or kidneys. Following intracerebral injection of the fetus, there is frequently a localization of the virus in the placenta. This oftentimes calls forth a maternal inflammatory response which breaks down the fetal epithelium and allows the liberation of infected fetal macrophages into the maternal circulation. Under such circumstances and when only a few old and well infiltrated lesions were present in the salivary glands of the mother, evidence of very recent infection of the renal epithelium was found in some of the mothers (Fig. 6), whereas signs of reinfection were not found in the kidneys of the mothers when their salivary glands contained well developed but relatively uninfiltrated foci of infection with the submaxillary gland virus.

Discussion

Unlike many of the naturally occurring viruses affecting laboratory animals, the submaxillary gland virus of guinea pigs appears to be thoroughly benign and of limited distribution in tissues. In a sense it bears a certain resemblance to the so-called "opportunists" among the normal bacterial flora of the body, in that it behaves as a pathogen only when fortuitously or artificially removed from its normal habitat and introduced into very vulnerable tissues such as the meninges or those of the fetus.

In this connection it is of interest to consider the distribution of intranuclear inclusions of unknown etiology in human salivary glands and other organs. The pertinent literature was summarized by Farber and Wolbach in 1932.[1] In the review of their findings, and those of other workers, intranuclear inclusions have been reported in the duct epithelium of the salivary glands in 35 of the 51 cases studied. All 35 were infants less than 2 years of age. Of the 17 cases in which inclusions were present in other viscera, 7 were either stillborn or premature and 1 was 2 days old. Except for 1 adult, none of the 17 was more than 2 months of age.

If it be presumed that the inclusions seen in these cases are of virus etiology, it follows that nearly half the individuals with visceral lesions acquired infection *in utero*. In addition to the striking morphological similarity of the inclusion bodies in both these human and guinea pig tissues, it is to be noted that the distribution and character of the lesions in the stillborn and premature group are remarkably like those we have described in the experimental infection of the fetus with the submaxillary gland virus of the guinea pig.[11] Moreover, the sites of the lesions in the 35 infants between 2 months and 2 years of age is in keeping with the localization of inclusions and virus in spontaneously infected guinea pigs.

Although many investigators have suspected the virus etiology of the inclusions in human salivary glands and other organs, to date there has been no report of successful isolation of a virus by animal inoculation. If such a virus exists and has properties similar to those of the salivary gland viruses of rodents, animal inoculation as a method of isolation is not likely to succeed because the viruses of guinea pigs, rats, mice and hamsters are

strictly species specific. Therefore, tissue culture of human tissues, such as those of the umbilical cord or of the stillborn, may offer a favorable medium for the isolation of the hypothetical virus.

Summary

1. The characteristics of spontaneous infection with submaxillary gland virus of guinea pigs are described. The incidence in various local stocks of guinea pigs was found to be from 7 to 74 per cent. Inclusions were found in the renal epithelial cells of 8 per cent of adult animals examined.

2. Histological evidence is presented which suggests that the inclusion bodies associated with the guinea pig salivary gland virus are composed of elementary bodies similar to those known to occur in certain other virus diseases.

3. The relative infectivity of the virus is greater for the fetus than for the postnatal guinea pig.

4. Natural passive immunization *in utero* or *per colostrum* is inadequate to protect fetuses or suckling guinea pigs against experimental infection. In spontaneously infected adults the quality or duration of active immunity may depend upon the presence of active lesions.

5. An analogy between the distribution of the inclusion bodies sometimes found in stillborn and premature human infants and in experimentally infected guinea pig fetuses is pointed out.

Note: I wish to express my appreciation to Dr. N. Paul Hudson for his advice and guidance throughout the course of this study, and to the donors of the Mr. and Mrs. Frank G. Logan Fellowship Fund.

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DESCRIPTION OF PLATE

PLATE 62

FIG. 1. Fresh, unstained wet mount from the meningeal exudate. The intranuclear inclusion appears as a granular mass in the center of the greatly distended nucleus. × 1450.

FIG. 2. Unstained glycerin mount of Zenker-fixed submaxillary gland. The hypertrophied cells lining the duct contain intranuclear inclusions which appear to consist of corpuscles or elementary bodies of uniform size. × 450.

FIG. 3. Longitudinal section of duct in submaxillary gland stained with hematoxylin and picric acid. The arrow indicates an inclusion in which the elementary bodies are arranged in concentric rings. × 450.

FIG. 4. Feulgen stain of infected duct cells in the submaxillary gland. The cytoplasmic inclusions stain deeply while the intranuclear inclusion has a delicately honeycombed appearance and stains less intensely. Altmann fixation. × 1000.

FIG. 5. Desquamated epithelial cell in renal tubule showing an intranuclear inclusion. Hematoxylin-eosin stain. × 1000.

FIG. 6. Intranuclear inclusions in the renal epithelium of a guinea pig whose fetuses were infected *in utero*. Eosin-azure stain. × 1000.

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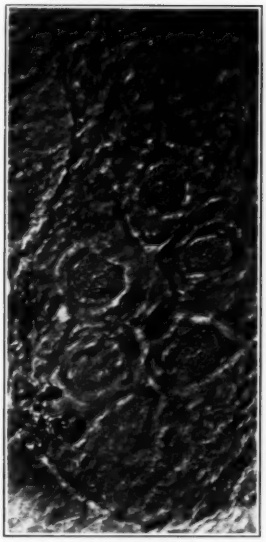

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Markham Submaxillary Gland Virus of Guinea Pig

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THE BONE AND CARTILAGE LESIONS OF PROTRACTED MODERATE SCURVY *

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TERMINOLOGY

In our opinion a great many of the words and expressions relating to conditions produced by vitamin C deficiencies are not well adapted to their purpose and in some cases are even incorrect. To justify the terminology used in this paper we shall briefly comment on this matter.

In the first place, the word *deficiency* refers to a concept that is hard to grasp. With regard to a vitamin, a state of deficiency exists in a diet that contains either none of the vitamin or any amount that is less than an adequate ration. More than the adequate amount results in excess. Thus, less than adequacy is deficiency and more than adequacy is excess, and both of these (deficiency and excess) can vary in extent; for instance, we can say a slight deficiency, or a great excess. However, a deficiency or an excess cannot exist in part so the commonly used terms "partly deficient" and "partial deficiency" are, strictly speaking, as meaningless as would be "partial excess" or "partly excessive."

The inaccuracies in the terminology of the conditions produced by various degrees of vitamin C deficiency are of a more serious nature than the foregoing. The word *scurvy*, being adopted before the cause of the disease was known, was originally defined by a clinical picture; to this a pathological picture was added later. The classical picture of scurvy is typified by the cases formerly arising on sailing vessels taking long voyages and in the first winter settlements of North America. This classical picture of the disease resulted from a great deficiency of the vitamin. But this is not the condition that interests us to-day, because it is seldom present. There is, however, a great deal of evidence [1-7] that indicates slight and moderate deficiencies are by no means uncommon. The condition resulting from these deficiencies (slight and mod-

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erate) has been called "subclinical scurvy," "the subscorbutic state," "latent scurvy," "chronic scurvy," and so on. Our reasons for objecting to the use of these terms are as follows:

In our opinion there is no need to restrict the name scurvy to the classical picture of the disease (hemorrhages, loosened teeth, aching muscles, prostration, and so on) which is the result of a great deficiency. Now that the cause of the disease is known, we think the fundamental definition should be a genetic one; we would prefer to say that scurvy is *the disease that results from a vitamin C deficiency*. Just as there are degrees of deficiency, so the resulting disease varies in degrees of severity. We suggest that the condition produced by a great deficiency be termed *severe* scurvy, and that those conditions produced by lesser deficiencies be termed *moderate* and *mild* scurvy. The terms mentioned above which are in use at present for these conditions are not adapted to their purpose. For instance "subclinical scurvy" is a misleading term because it implies that no clinical manifestations of the condition are present, whereas it may be that they have not yet been identified; "chronic scurvy" brings with it the implication of length of time, which is not always the point in question; "latent scurvy" would be applied more accurately if it referred only to the time that elapses before any indication of the deficiency is manifest; and "subscorbutic state" is an ambiguous sort of term.

Introduction

The diaphyseal lesions of severe scurvy have been carefully studied in both man and the experimental animal by several investigators. Little attention, however, has been focussed on the epiphyseal lesions of severe scurvy or on any lesion of the long bones in the milder forms of the disease. As slight and moderate vitamin C deficiencies have been shown to be not uncommon [1-7] it seems desirable to learn what bone lesions they would cause. Furthermore there is the possibility that a study of the lesions from mild scurvy might throw a different light on the action of the vitamin than do studies of the severe condition. Finally it is of interest to investigate the epiphyseal lesions of any scorbutic condition; these lesions, so far as we know, have not yet been described. Consequently an experimental study embracing these

points was undertaken, guinea pigs being used as experimental animals.

Material and Methods

Approximately 60 guinea pigs of mixed breeds and of both sexes were used. Each litter of 3 or 4 animals was put in a cage by itself at the age of 1 month. One of the animals of each litter was used as a control and the experimental procedure was begun at this time on the remaining 2 or 3 animals. The basal diet, fed all the animals *ad lib.*, consisted of a mixture of equal bulks of bran and rolled oats, 56 per cent (by weight); skimmed milk powder, baked in an open pan at 100–110° C. for 12 hours with occasional stirring, 30 per cent; butter fat freed from curd, 10 per cent; dried brewer's yeast, 1.5 per cent; cod liver oil, 1 per cent; sodium chloride, 1 per cent; and ferrous lactate, 0.5 per cent. This basal diet was tested as to its scorbutogenic properties at frequent intervals throughout the experiment by feeding it alone to guinea pigs and ascertaining that they died of severe scurvy in approximately 1 month. The control animals received in addition to this basal diet, 1 cc. of orange juice per 100 gm. body weight, plus an additional 1 cc. as a margin of safety; this is the minimum adequate ration proposed by Dann and Cowgill.[8] The experimental animals received in addition to the basal diet one of three different rations of orange juice, 0.5 cc., 0.75 cc. or 1 cc. per day. A ration once adopted was adhered to until the animal died or was killed.

The bones from one side of each animal were prepared for gross examination by drying them. The bones from the other side were prepared for microscopic study. For the latter purpose blocks that contained a joint were usually selected, in order that epiphyseal lesions might be studied. The material was fixed in Allen's fluid, decalcified in this fluid or in 5 per cent nitric acid, sectioned by the paraffin method and stained with hematoxylin and eosin.

Observations

Observations were made on five groups of animals: controls; those receiving no vitamin C; and three groups, each of which was subjected to a different degree of deficiency. We shall not describe the appearance of normal bones or of bones encountered in severe

scurvy, except for purposes of comparison. Furthermore, the three degrees of deficiency resulted in lesions which also varied only in degree. Therefore we shall not describe all three, but confine ourselves to the typical lesions encountered in the various parts of long bones of an animal suffering from a protracted moderate deficiency. For purposes of simplicity we will further confine our observations chiefly to those lesions seen in *growing bones*. This point is important because growing bones suffer from different lesions than mature ones.

Gross Findings

The long bones of the animals which grew under a handicap of deficiency were only slightly shorter than those of the controls but they were much lighter, dried specimens weighing only about three-fourths of similarly treated control bones. Furthermore, the bones of the experimental animals were fragile, rough and discolored, and they frequently exhibited fractures of the shaft near the epiphyseal plate. Many of the articulating surfaces were somewhat flattened and not uncommonly invaginated for a very short distance into the epiphyses.

Microscopic Findings in the Diaphysis

It is difficult to grasp the character and significance of the diaphyseal lesion of moderate scurvy unless one knows the function of the disc of trabeculated bone which exists in a normal growing bone on the diaphyseal side of the epiphyseal plate. During growth, as can be seen in Figure 2, the tubular shaft of compact bone does not extend to the epiphyseal plate; it terminates under the plate in a zone of trabeculated bone which has not yet become compact. Unlike the compact bone, this honeycomb of trabeculated bone is not limited to the periphery of the bony structure but extends across the medullary cavity and caps it. This is necessary because, since cancellous bone is not so strong as compact, there has to be more of it to afford the same support. In this location of active growth, however, it is admirably adapted to its purpose because it develops very quickly and thus serves as a strong and extensive temporary scaffolding until the more laboriously constructed compact bone can be consolidated in its periphery. When the periphery at any level is sufficiently

strengthened the central cancellous framework is there rendered unnecessary and is dispensed with. It is with this temporary scaffolding that the scorbutic lesions of the diaphysis are concerned; progressively greater deficiencies in vitamin C produce correspondingly greater deficiencies in the cancellous scaffolding and number of osteoblasts in this location.

In protracted moderate scurvy the formation of this cancellous scaffolding is affected far more at the central portion of the cap than at the periphery. A glance at the more centrally located portion of the epiphyseal plate and at the tissue on its diaphyseal side gives the impression that longitudinal growth has ceased, because there is scarcely any indication of bone formation in this region (Fig. 1). However, a glance at the periphery of the cap serves to correct this impression because in this location there are some indications of cartilage being replaced by new bony trabeculae. Thus, the chief effect of the subminimal ration is to diminish the number and activity of the cells concerned in building this temporary bony scaffolding; as a result of this, the amount of scaffolding constructed is likewise diminished and what small amount is constructed tends to be limited to the periphery of the bone. This allows the bone to continue growing in length, but because the amount of temporary scaffolding is diminished, the bone becomes exceedingly weak on the diaphyseal side of the epiphyseal plate, where we commonly found fractures.

Other findings of interest present were: (1) That portion of the epiphyseal plate which caps the marrow cavity was for the most part thinner than usual although in a few areas it was thicker than normal. The columns of cartilage cells in it were less regular than usual. (2) That portion of the shaft which normally consists of compact bone was not as dense or as thick as is normal. (3) Examples of Gerustmark (fibrillar or framework marrow) were not as common as they are in severe experimental scurvy. Even in specimens that showed very meager bone formation, Gerustmark was not necessarily a prominent finding.

Microscopic Findings in the Epiphyses

Articular cartilage presents an uncalcified zone towards the joint cavity and a calcified zone towards the layer of bone which underlies and supports it. This shell of cartilage and underlying

bone is normally supported by a scaffolding of cancellous bone. The uncalcified cartilage, the calcified cartilage and the supporting bone were all affected by protracted moderate scurvy. The supporting bone was diminished in amount and in some places there was none to support the cartilage (Figs. 3, 5 and 6). In some animals the zone of calcified cartilage encroached on the uncalcified so that a greater percentage of the articular cartilage was of the calcified variety. (This can be seen in Figure 3 where the calcified cartilage is deeper in color even than the underlying bone.) The uncalcified cartilage, in many sections, appeared thinner than normal.

The above stated fundamental changes were often accompanied by secondary changes. The weakened shell of cartilage and bone was often found distorted and fractured. Broken fragments of articular cartilage were found in masses of fibrous connective tissue which originated from the capsule. Capillaries were sometimes seen making their way into the uncalcified cartilage and these almost reached the surface in many instances. In some places where the cartilage had given way, the surface was covered with a layer or several layers of large cells with eccentrically located nuclei and basophilic cytoplasm. These cells resembled osteoblasts but were not associated with bone matrix (Fig. 6). The tips of the menisci in knee joints were often fibrinous in character; Figure 5 shows this, as well as some fibrinous exudate lying beneath the tip of the meniscus.

The connective tissue capsule of the joint, as well as the muscles associated with the joint, also showed lesions, a description of which is, however, outside the scope of this paper.

Comment

Character of the Bone and Cartilage Lesions of Scurvy

The term atrophy is commonly used in connection with the bones in scurvy. This does not necessarily mean that scurvy is a disease that causes bone resorption. For bone is one of those tissues like epidermis which is constantly being worn away and just as constantly replaced. Hence a bone can become atrophic, either through increased resorption or decreased restoration. It is important to decide which factor is involved in scurvy.

With this in mind, reference to growing scorbutic bones reveals

that the portion of the shaft laid down before the onset of the disease is affected much less than the tissue laid down after the onset of the disease. Thus the growing bone indicates that scurvy inhibits the formation of new bone rather than the speeding up of bone destruction. The chief lesions are located in the sites that are concerned with increasing the size of the bone, namely under the periosteum (where peripheral growth occurs), and in the metaphyses (where growth in length occurs). In investigating the effect of scurvy on growth we shall confine ourselves to its effect on that process which normally accounts for longitudinal growth. We shall do so because here the growth process and its disturbances are much more easily followed.

Our investigations into the effect of scurvy on longitudinal growth bring to light a paradox, namely that the bone continues to grow while very little new bone forms on the diaphyseal side of the epiphyseal plate. This calls for an explanation. The matter has been commented on by Park, Guild, Jackson and Bond [9] in their extensive report concerning their X-ray and histological studies on the scorbutic bones of children. They believed, if we interpret them correctly, that bones continue to elongate in scurvy because the cartilaginous epiphyseal plate, on which longitudinal growth depends, continues to grow. They believed, moreover, that this cartilage was not replaced by bone but accumulated on the diaphyseal side of the plate as a honeycomb of heavily calcified cartilage, which they called the "lattice." This heavily calcified lattice showed up readily in X-rays and so provided an excellent means to help diagnose early scurvy in children.

Our conception of the sequence of events that occur in the metaphysis and that allow growth of bone to continue in scurvy is somewhat different from that of Park, *et al.* As stated, we generally found that the epiphyseal plate was thinner and we did not find accumulations of calcified cartilage at the diaphyseal side of the plate. Therefore, we do not think that an accumulation of calcified cartilage is a correct explanation for the continued growth of bone in scurvy. We had many sections (and Fig. 1 is a good example) that showed almost no accumulation of cartilage and almost no new bone formation at the center of the diaphyseal side of the plate, and we reached the conclusion that the central part of the plate becomes more or less dormant in scurvy. We

think that under these conditions its diaphyseal aspect would become heavily calcified and so show up in X-rays, thus accounting for the "lattice"; this "lattice" would further be thrown into sharp relief, as Park, *et al.*, point out, because there would be so little bone on its diaphyseal side. We think that longitudinal growth of bone continues under these conditions because the plate, almost dormant in its central regions, is carried along by growth activity in its periphery.

If the supplies of growth-energy for forming new bone, which are apparently inadequate in scurvy, were distributed equally in all the places where new bone normally develops, there would be so little for each part that longitudinal growth would be very slight. Instead of this, Nature gives the greater part of her restricted ration to the periphery of the metaphysis, and she cuts down at those places, *e.g.* the center of the plate, where new bone would give strength but where it is not essential to growth. The periphery of the metaphysis is the only place where this slight ration would maintain the growth of bone. Hence, just as a normal bone is a striking example of nature's architectural abilities when she has an abundance of material, so scorbutic bones are witnesses to the fact that nature is equally capable of making the best of a bad situation caused by lack of workmen and building materials. The effect of scurvy on epiphyses has not, to our knowledge, been described. In general the same effects are found here as in the diaphysis; there is a marked diminution in the amount of newly formed bone and a slight interference with the normal growth of cartilage.

Growth versus Maintenance Lesions

The next point we wish to discuss is whether or not our experiments give any clue as to the effects of long continued mild vitamin C deficiencies on the cartilage and bones of an adult. It must be realized that there are no criteria at present available by which the pathologist can identify the effects of deficiencies in such cases — that is, in the bones and joints of adults who were subjected to a prolonged slight deficiency after they had attained full growth. Our results indicate that bone and cartilage formation are diminished by any scorbutic condition. This deficiency manifests itself in growing bones at the sites where the growth occurs.

But in adult bone growth is over and the only new bone and cartilage that forms in them is for the purpose of replacing breakdown. It is well known that the structure of bone is always changing; old Haversian systems constantly break down and are replaced by new; and Elliott,[10] in this laboratory, has recently shown that adult articular cartilage also gives evidence both of wear and of growth to replace wear. Consequently, in the adult we would expect to find the effects of long continued mild scurvy manifesting themselves not in special sites of growth, because there are none, but by general poor maintenance of the skeleton and articular cartilages caused by failure to replace breakdown. Curiously enough similar findings are characteristic of hypertrophic osteoarthritis. The bones in this condition become light and fragile. The term hypertrophic (referring to the presence of osteophytes) gives a false impression of increased bone growth; in reality there is far less bone in the skeleton than is usual. Having seen the way that nature uses her limited supplies of new bone in longitudinal growth, one would not be surprised to find that osteophytes are simply another example of nature's using her limited resources to best advantage. Osteophytes may be an attempt to prevent movement and thus protect both the cartilages and the bones. Others have already suggested a possible association between scurvy and rheumatic fever: we suggest furthermore the possibility of protracted mild scurvy (or a metabolic condition similar to that found in mild scurvy) being a factor in the defective maintenance of articular cartilages and bones so characteristic of osteoarthritis.

The Action of Vitamin C

In his recent review, King [11] states that "the two specific roles for vitamin C in animal tissues which appear to be clearly established are (*a*) respiratory function in serving as a hydrogen transport agent between unidentified metabolytes and other carriers of molecular oxygen, by way of two or more oxydase systems and (*b*) regulation of the colloidal condition of intercellular substances as shown by Wolbach and associates." As our findings did not suggest that vitamin C was concerned in the regulation of the colloidal state of intercellular substances, and as a careful study of the literature impressed us that the evidence for this

theory is far from satisfactory, we cannot agree with King that his function (*b*) of the vitamin is by any means clearly established. As a decision on this point is of no small importance, we therefore propose to review briefly the evidence that purports to support this theory and to discuss it in the light of our own and other findings.

The theory as stated by Wolbach and Howe [12] reads, "We therefore advance the theory that the failure of cells to produce intercellular substances in scorbutus is due to the absence of an agent common to all supporting tissues which is responsible for setting or jellying of a liquid product." We will henceforth refer to this as the "jellation theory" so as to avoid unnecessary repetition.

So far as we have been able to determine, the evidence for this theory is of two sorts.

1. That in scurvy there is an accumulation of fluid substance in both the bones and the teeth. This fluid substance is regarded as a secretion product of osteoblasts and odontoblasts respectively (unset intercellular substance) and it remains fluid in scurvy because of a lack of the jellation factor.

2. That in the healing of scurvy, firm intercellular substances form with such rapidity in the bones and teeth that the process is thought to indicate the jellation of previously elaborated fluid intercellular substance, the inference being that there would not be time for such extensive formation of completely new intercellular substance.

The evidence for the jellation theory obtained from (1) scurvy and (2) the healing of scurvy will now be subjected to scrutiny.

I. Evidence for the Jellation Theory from Studies on Scurvy

A. In the Bones: In 1919 Aschoff and Koch [13] observed that zones of normal active bone formation were replaced in scurvy by a marrow framework (Gerustmark) which contained little in the way of firm intercellular substance. They came to the conclusion that the primary defect in scurvy was a lack of a cement substance without which firm intercellular substance could not be made. In 1926 Wolbach and Howe studied the bones in scurvy and stated that they believed the loose texture of the Gerustmark to be caused by the presence of liquid intercellular substance. Thus Aschoff and Koch, and Wolbach and Howe visualize continued secretion on the part of

the osteoblasts in scurvy but a failure of their secretion to jell.

In contrast with this we found relatively few examples of Gerustmark in protracted moderate scurvy. At the same time, and in sites where there was no evidence of Gerustmark, we found great interference with bone formation. In these areas of scanty bone formation there was no evidence of the accumulation of a fluid intercellular substance (Fig. 1); on the contrary the picture indicated that there was a general depression of all the activities usually concerned in bone formation.

B. In the Teeth: From their studies, Wolbach and Howe thought they obtained evidence of unset jell formation in the teeth in scurvy. They pointed out that the pulp became separated from the dentin and they thought this to be caused by a secretion of fluid intercellular substance by the odontoblasts. We think it would be very difficult to rule out artefact as a cause of the pulp being separated from the dentin in a cross section of an incisor tooth. In support of our contention we point out that Höjer and Westin's [14] paper contains illustrations of cross sections of normal incisors with the pulp separated from the dentin and of scorbutic teeth with the pulp adherent to the dentin.

2. *Evidence for the Jellation Theory Obtained from Studies on Healing Scurvy*

A. In the Bones: Wolbach and Howe stated that their theory as a whole was supported by the promptness and volume of matrix formation following the administration of antiscorbutics. They found that after 3 days' administration of orange juice to a scorbutic animal bone matrix was formed, and that after 6 days there was a definite architecture of bone trabeculae.

We do not think that the formation of matrix and trabeculae in the time stated proves the existence of previously elaborated unset jell because Ham [15] has pointed out that, in the repair of fractures of normal animals, completely new bone matrix can be formed in 48 hours and bony trabeculae in 4 days.

B. In the Teeth: The speed with which new dentin appeared in the incisor tooth of a scorbutic animal given orange juice also made Wolbach and Howe think that the process was one of the setting of the previously elaborated unset jell which had caused the pulp to separate from the dentin. Fish and Harris [16] have re-

cently shown that this new dentin (variously called osteodentin, calcific scar tissue, secondary dentin and pulp bone) which appears in the pulp after antiscorbutics are given a scorbutic animal is a *normal* component of the tooth at certain levels; they have furthermore shown that it is a product of the pulp, and that it forms in the pulp from pulp cells and not between the odontoblasts and the dentin. As this material is a normal component of the pulp at certain levels, they point out that the interpretation of cross sections of incisor teeth is a very difficult matter. It should be remembered that Wolbach and Howe could not study the same tooth microscopically before and after the administration of orange juice. To avoid the difficulties caused by studies of cross section, Fish and Harris made their studies on longitudinal sections of molar teeth (the incisors curve in two directions and cannot be sectioned longitudinally). Fish and Harris' illustrations of scorbutic teeth show no accumulations of fluid between the pulp and the dentin which could represent unset jell formed continuously during the scorbutic period; on the contrary their illustrations show clearly that the calcific scar tissue which forms after the administration of antiscorbutics develops in the pulp just as it does normally near the wearing surface or after any injury to the tooth. Thus in the light of their work there seems to be no support for Wolbach and Howe's contention that the "new dentin" which forms on the administration of antiscorbutics represents the jellation of previously elaborated, unset jell.

In the light of the foregoing we conclude that the evidence for the jellation theory is decidedly inadequate. We think the lesions of bone and cartilage can be explained by a theory that postulates vitamin C to be somehow concerned in the metabolic processes of cells. Such a theory was offered by Höjer [17] who, after careful studies on the tissues in scurvy, postulated vitamin C to be necessary for the growth and activities of cells in general and specialized cells in particular. This theory seems to us to be in accord with what we would expect from what has been found out about the vitamin in the field of biochemistry.

Summary and Conclusions

Protracted moderate scurvy was produced in young guinea pigs by feeding them diets containing less than adequate amounts of

vitamin C. It was shown that longitudinal growth of bone continued under this regimen only because most of the limited amount of new tissue which formed was in the peripheral part of the epiphyseal plate and in the ring of diaphysis adjacent to the periphery of the plate. This resulted in a weak shaft in this location and an unsupported epiphyseal plate. The chief manifestation of scurvy in the epiphysis was found to be a diminution in the amount of the bone supporting the articular cartilage. It was pointed out that in the adult, when growth is over, the effects of scurvy would become apparent in sites where continual replacement of tissue occurs to compensate for wear and tear. It was suggested that certain features of osteoarthritis (the poorly maintained articular cartilages, the generalized diminution of the amount of bone in the skeleton and the osteophytes) would be not unlikely effects of a long continued moderate vitamin C deficiency in the adult. Lastly the theory which postulates that vitamin C controls the jelling of intercellular substances was discussed in the light of other and our findings, and no support was found for this theory.

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DESCRIPTION OF PLATES

PLATE 63

FIG. 1. Metaphysis of upper end of tibia from a guinea pig that received a normal diet for 1 month after birth, and thereafter 0.75 cc. of orange juice and no other vitamin C for 50 days, when it died. × 50.

FIG. 2. Comparable section from a control animal of the same age. × 20.

A comparison shows that the scorbutic bone lacks a normal trabecular framework in the center of the diaphyseal side of the epiphyseal plate; the marrow extends to the plate for there is absence not only of trabeculated bone, but also of calcified cartilage or semifluid Gerustmark in this location. At the periphery of the shaft enough new bone is formed to permit the shaft to increase in length. The shaft is very weak at this level, having almost no support of internal trabeculated bone.

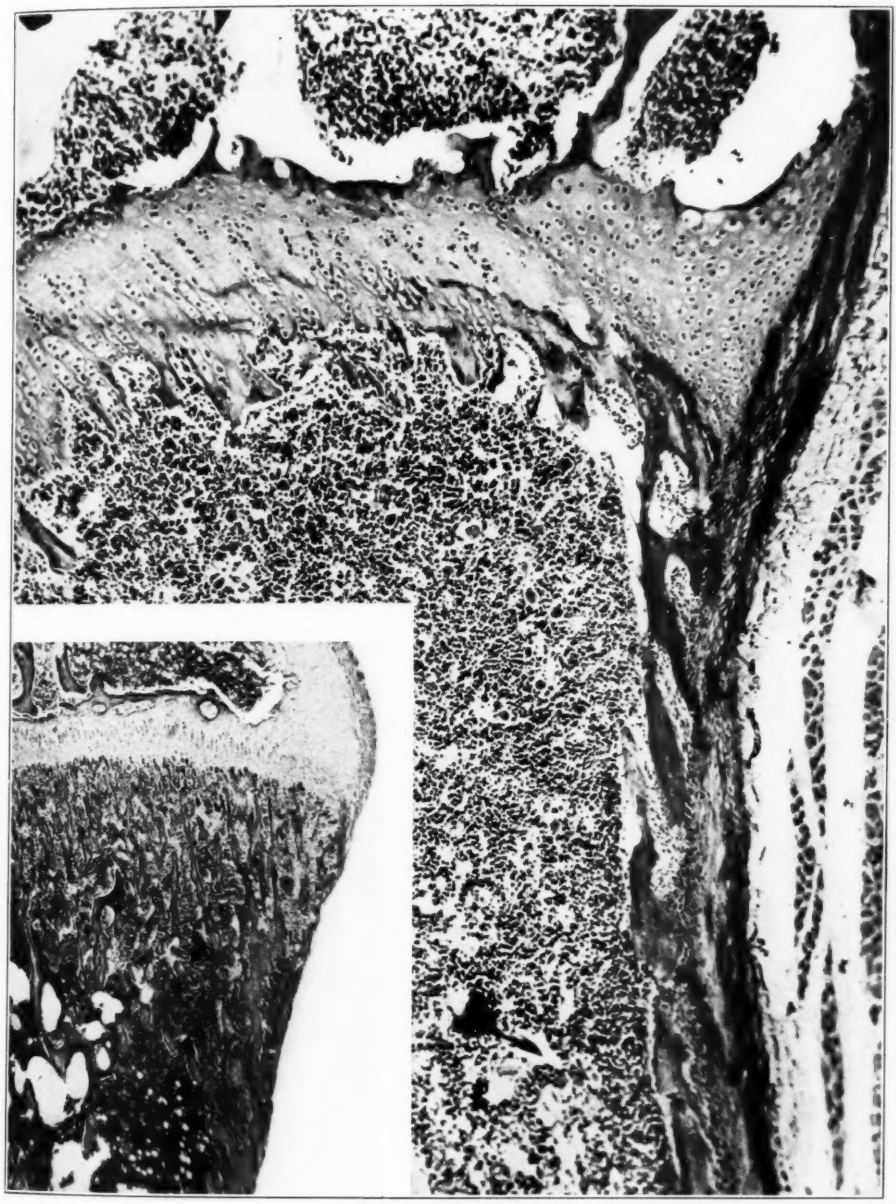

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PLATE 64

FIG. 3. Knee joint of a guinea pig that received a normal diet for 1 month after birth, and thereafter 0.5 cc. of orange juice daily for 120 days, when it died. × 80.

FIG. 4. Comparable section from a control animal of the same age.

A comparison shows that the scorbutic bone has a band of intensely staining calcified cartilage between the uncalcified cartilage and the supporting bone. The bone is lighter staining than the calcified cartilage, and is far less in amount than is normal. In a few places the cartilage is supported by no bone at all. The trabeculae of cancellous bone in the interior of the epiphysis are also fewer in number than is normal. This plate shows how protracted moderate scurvy diminishes the amount of bone that supports the articular cartilage.

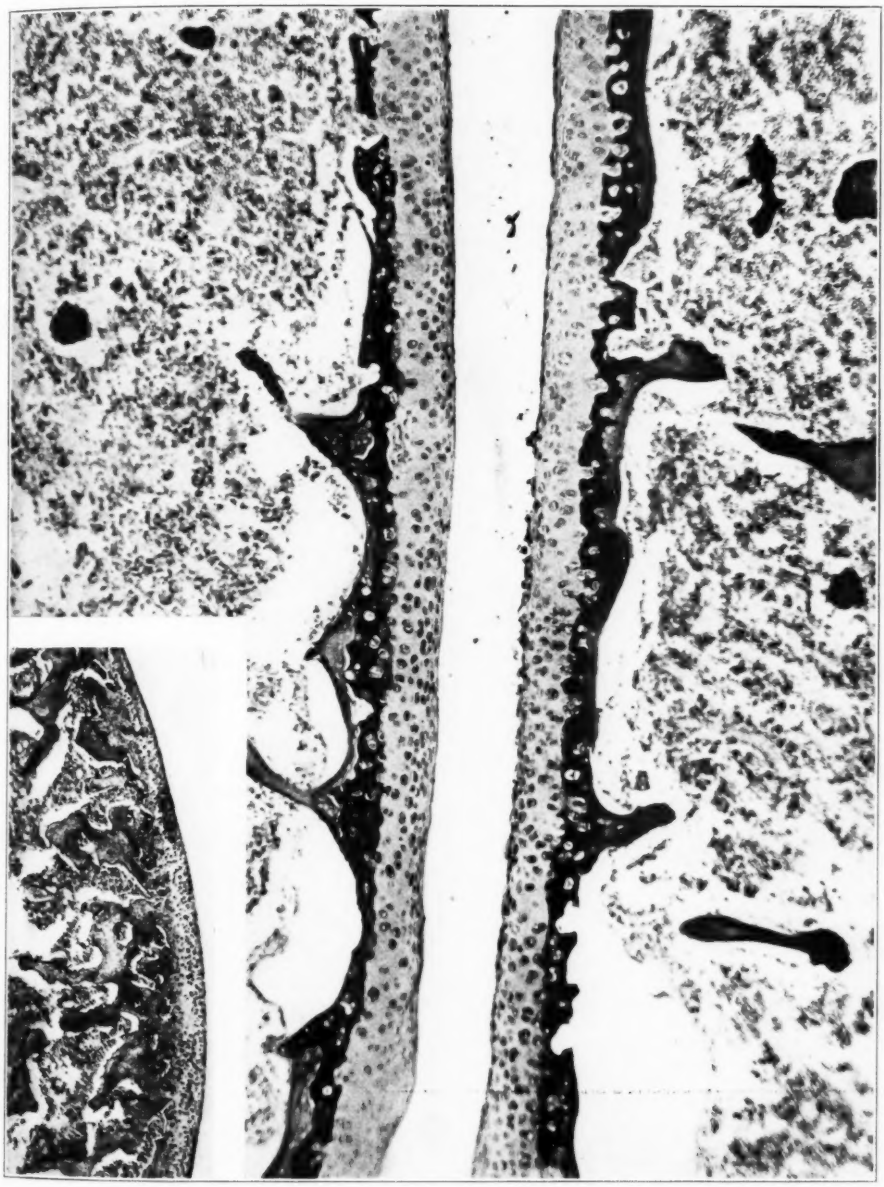

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PLATE 65

FIG. 5. Section of the knee joint of a guinea pig that received a normal diet for 1 month after birth, and thereafter 0.5 cc. of orange juice daily for 83 days, when it died. × 80.

The articular cartilage rests on almost no bony support, and in one location has given away (center of photograph). There is some fibrinous exudate directly above the fracture and the adjacent tip of the meniscus shows an inflammatory cell reaction. A few hyperchromatic nuclei at the site of fracture probably indicate a feeble attempt at repair.

FIG. 6. Section of articulating surface of the patella of a guinea pig that received a normal diet for 1 month after birth, and thereafter 0.5 cc. of orange juice daily for 120 days, when it died. × 80.

This section shows an older fracture of the articular cartilage. There is almost no supporting bone and the calcified cartilage (very dark stain) is broken up. The area in which the cartilage has given way contains a proliferation of cells which resemble osteoblasts or tendon fibroblasts; these can be seen on the surface. Deeper (between the fragments of cartilage in the center of the photograph) there is some fibrous tissue.

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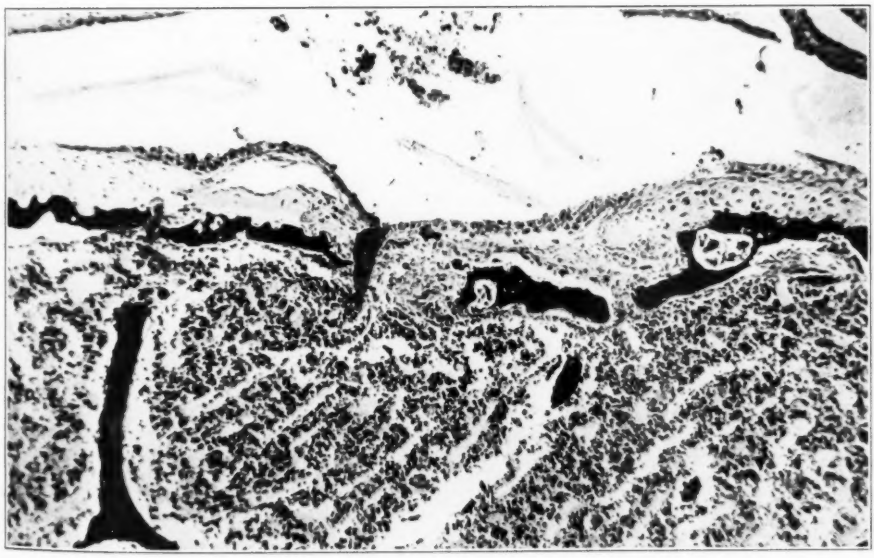

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TUBERCULOSIS IN ALLERGIC AND DESENSITIZED GUINEA PIGS *

A Study of Histological Changes

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Tuberculosis, as it develops in normal and allergic guinea pigs, has been studied extensively, the literature on the subject showing rather general agreement that animals rendered allergic by infection with tubercle bacilli of low virulence are protected to a certain degree from subsequent infection with fully virulent microörganisms. There is less general agreement, however, on the question of whether or not the allergic state must necessarily accompany this partial immunity. Rich,[1] in a series of experiments with various diseases, has indicated that desensitization of allergic animals is possible without subsequent loss of immunity. As far as tuberculosis is concerned, Rich's contention is based largely on the work of Rothschild, Friedenwald and Bernstein.[2] Cummings and Delahant [3] are the other workers in this country who have reported the treatment of guinea pigs with tuberculin in quantities sufficient to bring about complete desensitization. In both instances the desensitized guinea pigs were found to withstand subsequent virulent infection as well as the animals left in the allergic state. This contention is reinforced by the work of Yoshizawa [4] who treated tuberculous guinea pigs with a form of precipitated tuberculin.

Our own studies [5, 6] indicate that the three above-mentioned papers do not present the complete picture of the relation between allergy and immunity because the experiments were terminated too soon. In the case of Rothschild and coworkers all of the experimental animals had died or had been sacrificed 65 days after the virulent infection. Cummings and Delahant sacrificed their animals at the end of 6 weeks. Yoshizawa's animals were killed at

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varying intervals, but none was allowed to live longer than 50 days. During the first 2 months after virulent infection our findings were essentially similar to those reported by the above workers. In the desensitized animals the liver and spleen remained relatively normal in gross appearance while the lung showed nothing but miliary foci, usually widely scattered. During the 3rd and 4th months, however, the desensitized animals died from extensive tuberculous pneumonia. In every instance in which the experiment was allowed to continue for 6 months or longer, we observed a 100 per cent mortality in the desensitized animals long before all of the allergic control pigs had died. Yet even in those desensitized animals whose lungs were completely hepatized by tuberculous pneumonia, the liver and spleen were usually devoid of tuberculosis.

The extensive pulmonary disease in our desensitized guinea pigs, along with the lack of tubercle in the other viscera, seems the more remarkable in view of the well known predilection for tuberculous infection exhibited by the guinea pig spleen. As Krause has said,[7] "In guinea pigs . . . the spleen is undoubtedly the organ that is most prone to tubercle." And further, ". . . the guinea pig lung is highly resistant to infection. . . ." Our own experience with the development of tuberculosis in normal guinea pigs fully bears out Krause's statement. Nevertheless the fact remains that in the desensitized animals this "normal" picture is reversed, the lung becoming the seat of extensive disease while the liver and spleen are spared. This unusual phenomenon was deemed worthy of careful histological study.

As background for the comparison of tuberculous lesions in desensitized and control guinea pigs, we have had frequent occasion to refer to the above-mentioned paper of Krause, as well as to the studies of Willis.[8] Most useful, however, for obtaining an idea of histological changes in all the viscera has been the exhaustive description of tuberculosis in guinea pigs by Pagel.[9]

Experimental Material

Desensitized animals of two different types were available for study: (1) allergic-desensitized — guinea pigs rendered allergic by infection with the R_1 strain of bacilli, desensitized by daily

injections of tuberculin * and then reinfected with virulent tubercle bacilli; and (2) normal-desensitized — normal pigs given 1 cc. of old tuberculin daily, then infected with virulent bacilli. In both types of animals desensitization was maintained by continuing the daily injections of tuberculin in amounts of 1 cc. or more, the amount never being greater than 2 cc. daily.

Along with the two types of desensitized animals, control groups of two types were employed: (1) normal controls — guinea pigs normal at the time of the virulent inoculation; and (2) allergic controls — guinea pigs allergic due to R_1 infection at the time of the virulent inoculation. In most of our experiments young albino guinea pigs were employed, the animals weighing between 250 and 300 gm. at the time of inoculation. In all, more than 500 pigs were used in our various experiments.

The microörganism used for the virulent inoculation was our "G" strain of human type tubercle bacillus. Relatively large doses were employed, the dose for different experimental series varying between 0.3 and 1 mg. The animals were inoculated subcutaneously in every instance. It is of interest that even with these wide variations in the inoculating dose, typical differences in disease picture appeared between the desensitized and the control animals.

Most of our animals were allowed to live until death occurred. However, in two experimental series animals were sacrificed at biweekly intervals for a period of 10 weeks in order to determine the number of tubercle bacilli in the spleen of each animal. As described in detail elsewhere [6] this determination was made by culturing weighed amounts of each spleen according to the technic of Lurie.[10] The results of these cultures indicated a fairly consistent difference in splenic content of bacilli in the three groups of animals that were employed. The allergic animals showed the smallest count, averaging less than 5000 bacilli per spleen. The allergic-desensitized animals stood between the allergic and the normal controls, averaging about 100,000 bacilli per spleen, while the normal controls averaged more than a million bacilli per spleen.

For our histological study tissues from lungs, liver, spleen and kidneys of animals in all of the series were fixed in formalin and

* The tuberculin used in these experiments was furnished by Parke, Davis and Company, Detroit, Michigan.

stained routinely with hematoxylin and eosin. When indicated, Ziehl-Neelsen's and van Gieson's stains were employed also.

Observations

Lungs

Control Animals: Typical tubercles, in the sense of circumscribed nodules of concentrically arranged epithelioid cells, are not found in the lungs of infected guinea pigs (see also Pagel). The pearly nodules, miliary in size, so readily seen in the gross invariably reveal themselves under the microscope as heterogeneous accumulations of epithelioid cells, lymphocytes, large mononuclears and a few polymorphonuclear leukocytes (Fig. 2). Desquamated alveolar lining cells filled with lipoid droplets play a part in completing the obliteration of all the air spaces in these small areas. During the later months of infection control animals of both the normal and the allergic groups may show areas of bronchopneumonia.

Desensitized Animals: Early in the 2nd month of infection miliary lesions appear in the lungs of the normal-desensitized guinea pigs. Similar lesions are found in the lungs of the allergic-desensitized pigs during the 3rd or 4th month. If the animals are maintained in the desensitized state until death occurs, the lungs, at autopsy, are distended, firm, and are dotted with large white areas of bronchopneumonia (Fig. 1). Histologically the lesion is seen to be largely interstitial in nature. In the less involved areas strands of solidified pulmonary tissue alternate with distended emphysematous alveoli (Fig. 3). In these solidified strands the interstitial tissue is infiltrated with polymorphonuclear leukocytes, lymphocytes and large mononuclear cells, and such alveoli as may be incorporated in the strand are obliterated, usually due to metaplasia of the alveolar epithelium to a cuboidal type. In the more extensively involved portions the interstitial tissue over large areas is infiltrated and most of the alveoli are obliterated in the manner just described. In addition, the terminal bronchioles and a few of the alveoli are found filled with dense plugs of exudate made up largely of granulocytes but containing also large mononuclear cells and desquamated epithelial cells (Figs. 4 and 5). Only rarely does one find definite caseation with destruction of the

connective tissue framework of the lung. Sections of the lung stained with carbol fuchsin reveal numerous acid-fast bacilli throughout the areas of interstitial pneumonia, and, in the bronchiolar plugs, the bacilli appear in great clumps as though here the microörganisms had found ideal conditions for growth (Figs. 6 and 7).

In addition to the histological sections we have examined numerous impression smears made directly from a cut surface of the fresh lung and stained for acid-fast bacilli. These smears reveal that some of the acid-fast microörganisms are within polymorphonuclear leukocytes (Fig. 8) while others are in cells of the mononuclear series. Occasionally, however, great clumps of the bacilli are found as though growing freely, without any suggestion of phagocytosis. Occasionally the smears contain non-acid-fast diplococci, but these are relatively few in number.

The large number of acid-fast bacilli in the desensitized lungs is the more striking when compared with smears from control pigs stained by Ziehl-Neelsen's method. Even though the lungs of the latter animals are dotted with tubercles, a tedious search of sections or smears may reveal no acid-fast microörganisms whatsoever or, at most, only an occasional bacillus.

Liver

Control Animals: By the end of the 1st month the liver of the primarily infected control animal is almost invariably dotted with minute white foci. Under the microscope these miliary foci are seen as masses of epithelioid cells grouped mostly about the portal triad, though some are midzonal in distribution. An occasional giant cell may be seen and there is a slight infiltration with lymphocytes and granulocytes. With the lapse of time the periportal lesion continues to increase until, by the end of the 2nd month, the hepatic lobules are often completely outlined peripherally by tuberculous tissue.

Allergic control animals which have lived for several months show scarring of the liver, with connective tissue proliferation but no tubercle formation. The cirrhotic process is invariably of the portal type. Occasionally, in the larger areas of scarring, we have noted the proliferation of bile canaliculi.

Desensitized Animals: As already mentioned, the liver of the

desensitized guinea pig appears free from tubercles in gross. It is flabby in consistence and smaller in size than the liver of a comparable control animal (Fig. 1). The histological sections show atrophic parenchymatous cells and slight lymphocytic infiltration in the periportal region but no tubercles or tuberculous tissue. Occasionally the liver may show a small infarcted area.

Spleen

Control Animals: One week after infection of the normal control the malpighian bodies of the spleen show a slight polymorphonuclear cell infiltration. During the 2nd week epithelioid cells begin to fill the center of each malpighian body (Fig. 9) and, during the 2nd month, the normal lymphoid tissue becomes almost completely replaced by epithelioid cells. Spleens of the allergic controls exhibit a few conglomerate tubercles until several months have elapsed. Then they may show extensive fibrosis of the splenic pulp.

Desensitized Animals: The spleen of the desensitized guinea pig remains practically normal in size and exhibits no gross evidence of tubercle formation. By the 4th week after infection it shows, histologically, a fairly well marked band of tissue at the periphery of each malpighian body (Fig. 10). This band appears more eosinophilic than the surrounding splenic pulp and is demarcated rather sharply from the lymphoid cells of the malpighian body. When examined under high magnification the band is seen to be made up of reticulum cells in which there is a slight infiltration of polymorphonuclear leukocytes and a considerable accumulation of red blood cells. By the end of the 2nd month this band appears more dense and fibrous, a fairly definite perimalpighian fibrosis being the end result. A further change which occurs at these later intervals is thickening and fibrosis of the walls of the splenic sinusoids.

In the spleen, then, an initial perimalpighian lesion in the desensitized animal contrasts with the initial appearance of a central lesion in the malpighian bodies of the control animal. The lesions progress with time to the development of perimalpighian fibrosis in one case and to the almost complete replacement of lymphoid tissue with epithelioid cells in the other case. It should be mentioned again that, in spite of the absence of gross or microscopic

tubercles in the desensitized spleens, our bacteriological studies revealed the presence of large numbers of tubercle bacilli.

Kidney

Krause [7] has stated that "kidneys (of guinea pigs) practically never show gross tubercles"; and our own experience corroborates this statement. Nevertheless, in the present experiment the kidneys of all animals were subjected to careful scrutiny, not so much in the expectation of finding tubercles as to observe whether or not renal damage had occurred in the desensitized group from the excretion of tuberculin. We were not surprised, therefore, when definite renal lesions came to light in the desensitized animals, but we were not prepared for the finding of frequent lesions in the control kidneys as well.

No tubercles were found in the kidneys of either control or desensitized animals. However, in the majority of animals of both types, which lived as long as 2 months after infection and which were allowed to die from progressive tuberculosis, sections of the kidney revealed small cortical scars. In these scarred areas, involved glomeruli showed thickening and hyalinization of the glomerular capsule as well as of the glomerular capillaries. Frequently adhesions were found between tuft and capsule. The surrounding tubules were atrophic, the area being infiltrated with lymphocytes and large mononuclear cells (Fig. 11).

Lesions such as those just described were found with approximately equal frequency in the kidneys of desensitized and control pigs, the chief difference between the two groups appearing in the extent of tubular involvement. In many of the desensitized animals the convoluted tubules were extensively damaged.

Blood Vessels

In both control and desensitized guinea pigs that have lived for 8 weeks or more after virulent infection we have frequently noted vascular lesions. In the vicinity of the cutaneous ulcer caused by the primary inoculation the arterioles may exhibit an obliterating endarteritis (Fig. 12). More commonly, however, branches of the pulmonary artery and of the portal vein are involved. In animals that have been allowed to die from their disease the pulmonary arterioles are frequently found with the lumen completely oc-

cluded on account of contraction of the smooth muscle of the media. Hemorrhage in the surrounding pulmonary tissue indicates that this occlusion was an ante mortem rather than a postmortem occurrence. In the liver a similar marked contraction of the vessel wall is frequently found in branches of the portal vein.

Discussion

Histological differences between the lesions in control and desensitized animals are much as one might expect to find them from the variations in the gross picture. The most pronounced difference is found in the lung where the "tubercle" of the control animal, a more or less circumscribed accumulation of cells, gives way in the desensitized animal to an interstitial pneumonia which comes to involve a major portion of the entire lung. An even more striking difference exists in the relative number of acid-fast organisms — very difficult to find in the lungs of the control pigs but innumerable in smears and sections of desensitized lungs.

It is interesting to speculate regarding the possible cause or causes of this great difference in bacillary content of the lungs. As has been pointed out elsewhere,[6] the desensitized animals become markedly emaciated in comparison with the controls. They usually develop cutaneous ulcers which may persist for weeks. Furthermore, we have noted that after the injection of tuberculin each day the animals refuse to eat for several hours. They frequently leave portions of lettuce or other food untouched in the cage, with a resulting difference in the amount of vitamin intake as between the control and desensitized animals.

The inanition, emaciation, and ulcers of the skin all might be factors in bringing about the difference between the lungs of control and desensitized pigs. However, the one most important difference between the two types of animals is the difference in their allergic state, and this, we believe, may explain the observed changes. As Long [11] has so ably pointed out, tuberculosis, along with the other mycobacterial infections, is primarily a disease of cells of the monocytic type. Under ordinary conditions the guinea pig, following the development of allergy, seems enabled to exercise a certain amount of restraint on the growth of acid-fast microörganisms. But in the desensitized animal this restraining influence, at least in the lungs, seems to be lost completely. The

acid-fast bacilli appear there in great masses as though growing freely on excellent culture media.

A free proliferation of tubercle bacilli suggestive of that appearing in the desensitized lung has been observed in the guinea pig under entirely different conditions. It has been noted that tubercle bacilli, after intraperitoneal injection in normal guinea pigs, grow freely for a few days in (or on) the cells of the omentum.[12] It has been found further that this free growth period terminates in most of the animals 7 days after inoculation — at about the same time allergy begins to develop. Inasmuch as no free growth period was observed following the inoculation of timothy grass bacilli it was suggested that the capacity to grow freely for a period in guinea pig tissue is characteristic of virulent as distinguished from avirulent acid-fast bacilli and that protection from the overwhelming growth of virulent bacilli is a function of allergy.

It would seem that a comparison might legitimately be drawn between the above-mentioned work and the present study. In both instances we are dealing with non-allergic animals. Perhaps, in the lungs of the desensitized guinea pigs the tubercle bacilli, relieved of the restraining influence of allergy (and immunity), exhibit a phase of free and unrestrained growth comparable to the free growth in the omentum of the newly inoculated, non-allergic guinea pig.

Summary

A study of histological sections from the organs of desensitized guinea pigs reveals extensive disease of the lungs with an overwhelming accumulation of acid-fast bacilli, while the liver and spleen show no definite tubercle formation.

Lesions in the kidneys and blood vessels of both desensitized and control animals are described.

It is suggested that the lack of allergy is responsible for the free and unrestrained growth of tubercle bacilli in the lung of the desensitized guinea pig.

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DESCRIPTION OF PLATES

Figures 2–5 and 9–12 are from sections stained with hematoxylin and eosin. Figure 6 is from a section and Figures 7 and 8 are from impression smears stained with carbol fuchsin-methylene blue.

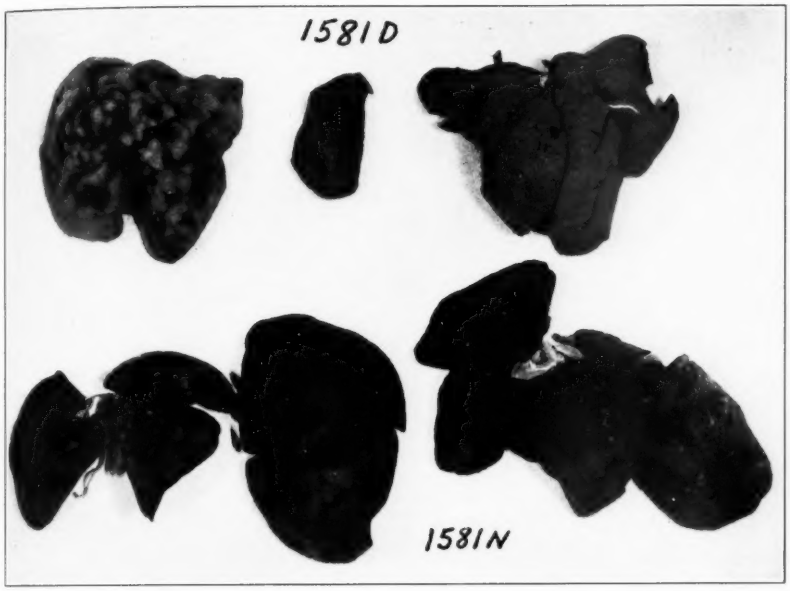
PLATE 66

FIG. 1. 1581 D. Lung, spleen and liver of *normal-desensitized* guinea pig 8 weeks after virulent infection. Note extensive involvement of lung while spleen and liver show no tubercles.

1581 N. Lung, spleen and liver of *control* guinea pig 8 weeks after virulent infection. The spleen is greatly enlarged and both spleen and liver show tubercles. Involvement of lung is less extensive than in 1581 D. Three-fourths natural size.

FIG. 2. "Tubercle" from lung 1581 N. of Fig. 1. × 90.

FIG. 3. Strands of solidified pulmonary tissue from less involved portion of desensitized lung. × 150.



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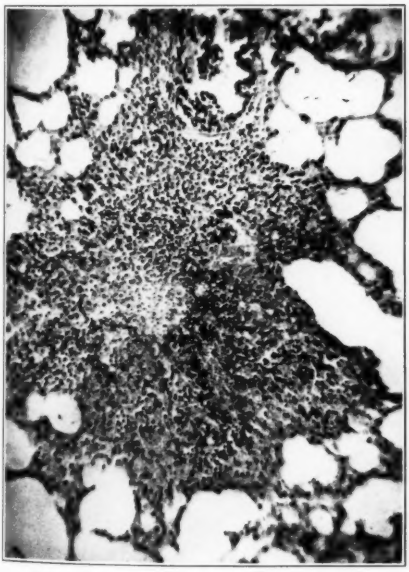

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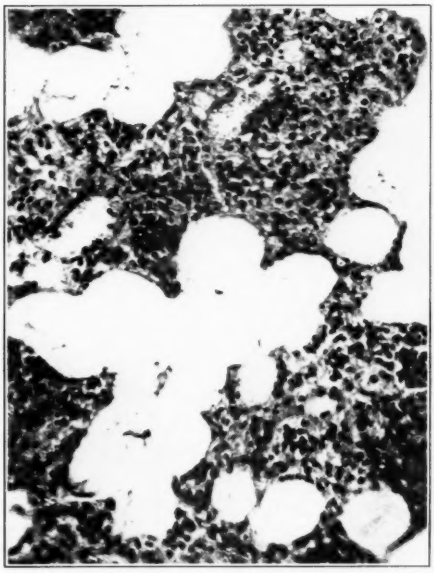

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PLATE 67

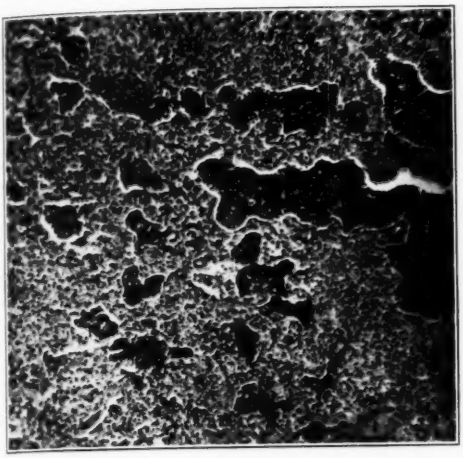
FIG. 4. Type of pneumonia that characterizes the lungs of desensitized animals dying from tuberculosis. The terminal bronchioles and certain of the alveoli are filled with dense plugs of exudate. This microphotograph is made from a section of lung 1581 D. × 75.

FIG. 5. Enlargement of one of the bronchiolar plugs shown in Fig. 4. These plugs are made up of polymorphonuclear leukocytes, large mononuclear cells and desquamated epithelial cells along with great masses of tubercle bacilli. Note how the plug, during fixation, has pulled away from the wall of the bronchiole, leaving a wavy white space at each side of it. × 230.

FIG. 6 One of the plugs of exudate within an alveolus. The photograph shows the acid-fast bacilli, only a few of which are in focus. Photograph taken with blue light. × 600.

FIG. 7. Impression smear made from cut surface of lung 1581 D, showing the large clumps of acid-fast bacilli. Photograph taken with blue light. × 600.

FIG. 8. Enlargement of a portion of the same smear. Each of the four clumps of bacilli illustrated is within a single polymorphonuclear leukocyte. Photograph taken with blue light. × 1500.



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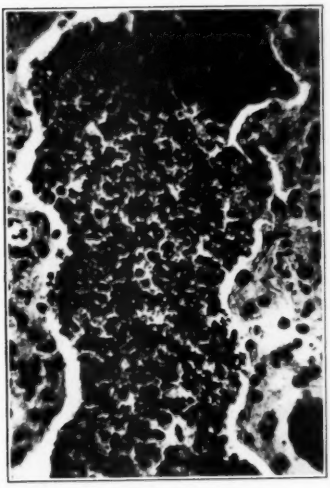

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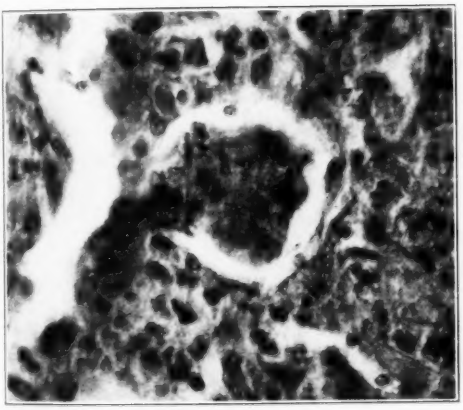

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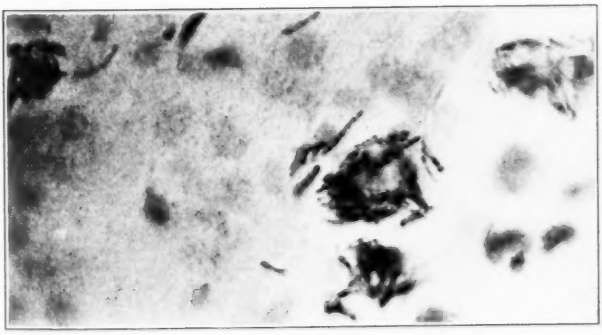

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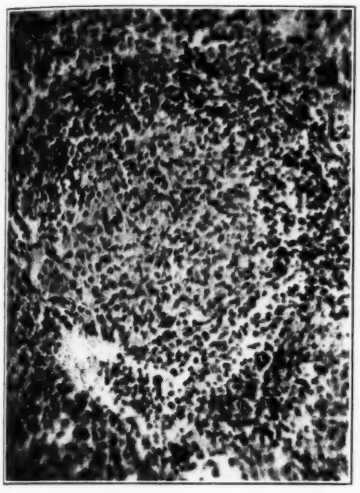
PLATE 68

FIG. 9. Malpighian body from spleen of control guinea pig 2 weeks after virulent inoculation. Note epithelioid cells in center of the malpighian body. × 120.

FIG. 10. Two malpighian bodies from spleen of allergic-desensitized guinea pig 4 weeks after virulent inoculation. Note the light band (eosinophilic tissue) at the periphery of malpighian bodies. × 75.

FIG. 11. Small renal scar in kidney of control guinea pig 8 weeks after virulent inoculation. × 230.

FIG. 12. Obliterating endarteritis in small artery found in vicinity of the cutaneous ulcer caused by primary inoculation with tubercle bacilli. × 350.



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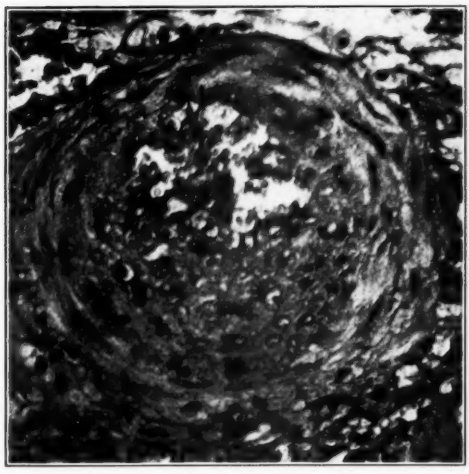

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FATTY INFILTRATION OF THE LIVER AND THE DEVELOPMENT OF CIRRHOSIS IN DIABETES AND CHRONIC ALCOHOLISM *

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Direct evidence that alcohol can cause cirrhosis of the liver has been demanded before it is accepted as the primary etiological agent in so-called alcoholic cirrhosis, though everyone knows that the association of the two for centuries has been that of a cause and effect relationship. The mechanism by which the cirrhosis develops has not been understood, and the introduction and acceptance of the terms atrophic and Laennec's cirrhosis as including all types that are obviously not biliary in origin have allowed confusion to prevail. Mallory in 1911 [1] pointed out the differences in anatomical types of cirrhosis but there is still too little effort on the part of pathologists and clinicians to distinguish these types. The writer pointed out in 1927 [2] that little could be learned about alcoholic cirrhosis of the liver, or any other kind, until we learned to classify them as to origin, progress and end result. There is no reason why I should enter into the endless discussion as to whether alcohol can or cannot cause cirrhosis of the liver. I wish to describe in this paper how it may cause it by first producing a fatty liver.

One of the first direct observations on the finding of fatty livers in subjects known to have been excessive drinkers of spirits was recorded by Rokitansky in 1849.[3] Previous to his report Louis in 1846 [4] had observed fatty livers in one-third of his cases of death from phthisis. Underlying this effect from two apparently widely different conditions there exists a very similar physiological process, as we shall see. Virchow [5] regarded a fatty liver as pathological and raised the question of fatty infiltration versus fatty degeneration, which has been a subject for discussion ever since.

* The experimental work cited in this paper was supported by the Christine Breon Fund for Medical Research.

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Saikowsky, in 1865,[6] is the first reference I can find to studies in which fatty livers were produced in experimental animals. By feeding phosphorus to three rabbits he found the fat content of the liver increased from a normal of 5 to 6 per cent, to 8, 12.5 and 11 per cent, respectively. By this analysis it was shown that fat infiltration in the strict sense occurred. In 1883 Lebedeff [7] showed again that the fat accumulation in the liver in phosphorus poisoning was the result of actual transport or migration into that organ.

In 1870 experiments by Ruge [8] designed to show the effect of alcohol on dogs showed incidentally that diet influenced the results considerably; that alcohol-treated animals on a milk or meat diet only, accumulated fat in the liver up to 63 per cent of their weight. When bread was added to the diet fat did not increase to the same extent in the liver. Rosenfeld in 1902 [9] pointed this out later and established the fact that starvation is of primary importance in the development of fatty infiltration under experimental conditions. Statkewitsch in 1894 [10] had described fatty changes in the muscles and liver in fasting and wasting diseases, such as typhoid, and mentioned that of all glands the liver is the most affected. This was essentially a starvation effect. While there have been other similar observations in man and experimental animals, Mottram's [11] report in 1909 proved the same thing in another way. By starving guinea pigs, rabbits, dogs, pigeons and cats he found that fat accumulated with astonishing rapidity in the livers, this showing a marked increase in as short a time as 24 hours. That the fat came from the normal fat depots was shown by the change in the iodine number of the liver fat. This (iodine number) decreased progressively, approaching that of normal fat, as the absolute amount of fat in the liver increased.

With few exceptions the infiltration of fat into the liver may be considered pathological. Virchow quotes Kölliker's observations that fat accumulated in small amounts in the livers of suckling animals. Coope and Mottram [12] noted some increase of fat in the liver during pregnancy and lactation in rabbits. Neutral fat may be found in small amounts after a meal containing a great deal of fat, but it is probable that in most other instances where neutral fat may be demonstrated, grossly or microscopically, the condition is due to some interference with normal carbohydrate-fat catabolism. The large number of conditions in which fatty infiltration may

occur or be produced experimentally include the wasting diseases, tuberculosis and malignant tumors particularly, in which there may be a demonstrable lipidemia; a diet in which carbohydrates are decreased to a minimum or are absent (meat diet in an herbivorous animal, Pagès [13]); and even to some extent in subjects who have been dying slowly for several days and either not eating or not assimilating food. The histological picture in the liver when this has occurred is a later stage of that usually observed in such cases, which is simple glycogen depletion. Likewise such poisons as ether, chloroform, carbon tetrachloride and alcohol definitely interfere with carbohydrate metabolism, and so with the proper oxidation of fat (Quastel,[14] Lattes,[15] Peters and Van Slyke,[16] and Goldschmidt, Ravdin and Lucké [17]). The moderate fat infiltration of the liver found in vitamin B_1 deficiency is probably due also to the influence this substance has on the proper oxidation of carbohydrates (Peters [18]).

That dietary insufficiency is in part responsible for fatty infiltration in alcoholism has been shown many times both clinically and experimentally. Among recent clinical observations that severe alcoholism and nutritional deficiency are coincident are those of Romano [19] in which 79 per cent of 131 alcoholics had had an inadequate food intake; of Patek [20] who found a significant relation between nutritional deficiency and alcoholic cirrhosis of the liver which was benefited by a high vitamin diet; and of Goodhart and Jollife [21] who found the same conditions and also described a case in which in addition to polyneuritis, "cirrhosis" of the liver was present. Observations reported here are simply repetitions of this not generally appreciated combination of pathogenic factors.

That alcohol alone will produce fatty infiltration of the liver was shown years ago by Ruge, and is asserted by Leathes and Raper.[22] Bollman and Mann [23] found that it increased the rapidity and degree of infiltration in dogs fed a high fat diet. That alcohol produces its effect by interference with tissue oxidation is shown by Higgins [24] on the effect of alcohol on tissue respiration, and Peters and Van Slyke [16] include alcohol among the poisons that produce a "histotoxic anoxia." Barcroft,[25] McFarland and Barach,[26] and van Wulfften Palthe [27] have commented on the anoxemia present in alcoholic intoxication, and the latter showed

that death from an otherwise lethal dose of alcohol could be prevented by oxygen inhalation.

Himwich *et al.*[28] concluded that the altered metabolism, which they found after alcohol administration (increase of lactic acid, increased carbon dioxide, change of pH to acid side, and increase of sugar in the blood) was due to an inhibitory effect on cellular respiration.* These effects are similar to those produced by ether, chloroform and carbon tetrachloride, and it is noteworthy that the experimental cirrhoses of the liver which most nearly approach alcoholic cirrhosis were produced by carbon tetrachloride (Lamson and Wing,[29] Bollman and Mann [30]), which has a depressant action on carbohydrates similar to alcohol, but a much more drastic action on the liver; and by phosphorus (Mallory [31]), which causes a fatty infiltration, as well as degeneration, of the liver.

An example of how the withdrawal of dextrose from body metabolism may result in a fatty liver is found in the effects of phlorhizin poisoning. Rosenfeld [9] showed that fat accumulated very rapidly in the liver (within 48 hours) when animals were treated with phlorhizin, and disappeared as rapidly. It was he also who pointed out that diet influenced the results of such experiments, as well as those with alcohol. I found that guinea pigs injected with phlorhizin did not develop fatty livers as long as they were fed a normal diet but did accumulate fat in the liver within 24 hours when starved for a day before treatment was begun.† Phlorhizin not only withholds sugar by causing rapid excretion, but actually withdraws glycogen from the liver and muscles so that again the proper breakdown of fat cannot take place.‡ The point I wish to make by this review is that fatty infiltration of the

* It is usually asserted because of the marked lowering of the respiratory quotient when alcohol is being oxidized that alcohol has "taken the place of carbohydrate" or has a "sparing action on sugar." Higgins (*loc. cit.*) says that probably 20 to 40 per cent of the total metabolism in alcoholism is due to alcohol. There is no evidence that alcohol can replace sugar and perform the same function as sugar in metabolism. The lowered respiratory quotient must mean simply that the demand for the oxidation of alcohol is imperative, and that metabolism is diverted for a short or for a long time from its normal carbohydrate-fat oxidation function to that of ridding the body of a poisonous agent.

† Unpublished experiments.

‡ The antagonism between glycogen and fat in the liver was pointed out by Rosenfeld (*loc. cit.*) and the statement has been repeated many times (see Lusk, G., Science of Nutrition, W. B. Saunders Company, Philadelphia, 1928, 310). This term is unfortunate as regards its chemical implications, but it does express the inverse relation between glycogen and fat in the liver in many conditions.

liver nearly always depends on the fact that carbohydrate, for one reason or another, becomes unavailable for tissue oxidation.

Fatty Infiltration and Cirrhosis in Diabetes

Fatty enlargement of the liver in diabetes mellitis has been observed casually for many years although reports are few. Pepper in 1884 [32] mentioned fatty accumulation and congestion of the liver but attributed this in part to intemperance. Pflüger in 1905 [33] observed that the liver contained more fat than normal in partially depancreatized dogs with diabetes. Umber 1914 [34] makes a terse statement to the effect that the liver is generally enlarged in diabetes and that this may be due to hyperemia, fat infiltration or incipient cirrhosis. Hanssen in 1936 [35] found enlargement of the liver in juvenile diabetes and states this must be due to fat. This could be reduced by proper diet and insulin treatment. Cruickshank in 1915 [36] demonstrated that fat loss in feces could be diminished by feeding pancreas to depancreatized dogs, and since then the fatty infiltration of the liver associated with pancreatectomy and its partial control has been discussed by the Toronto group (Allan, Bowie, Macleod and Robinson 1924,[37] and others), and by Chaikoff.[38] By prolonging the lives of depancreatized dogs it was possible to watch these fatty livers develop into a genuine portal cirrhosis of the liver similar to that found in man in diabetes and in alcoholism (Chaikoff, Connor and Biskind [39]).

I wish to report briefly 2 cases of cirrhosis associated with diabetes which came to autopsy.

Case 1. A male, 77 years of age, had had diabetes for at least 17 years. It was diagnosed after he had developed an intractable ulcer on a toe. He had lost weight from 185 to 145 pounds. With insulin and diet he became well enough to dispense with insulin after several months. During the course of his illness he neglected his diet a number of times and drank some wine. Gangrene of both feet developed again, which progressed slowly, and it was 4 months after this that he came into the University of California Hospital. He had palpable arteriosclerosis with sloughing gangrene of the 1st, 2nd and 3rd toes of the left foot. The blood pressure was 120 systolic, 80 diastolic. He died 3 days after admission, following a period of coma with marked ketosis and a blood sugar of 328 mg. per cent.

At autopsy the peritoneal cavity contained small amounts of fluid in scattered fossae. There was no fluid in the pleural cavities

but the lungs were congested and the heart weighed 580 gm. The aorta showed only a moderate amount of atheromatous degeneration with remarkably little calcification. However, the peripheral arteries showed advanced medial calcification.

The liver weighed 1720 gm. It was firm in consistence, had a granular irregular surface, and the cut surface was paler than usual. On microscopic examination fatty infiltration was marked and perilobular fibrosis prominent. The portal areas were irregularly thickened and showed some bile duct proliferation and scanty infiltration with lymphocytes. The picture was that of a typical portal and fatty cirrhosis (Fig. 1).

CASE 2. A male, 62 years of age, was known to have had diabetes for 6 years. This was fairly well controlled with 10 units of insulin daily. On occasions this had to be increased because of dietary indiscretions. There was no history of intemperance so far as alcohol is concerned. The liver was said to have been enlarged 8 months before admission to the University of California Hospital. The patient entered the hospital the day after he had vomited about 300 cc. of fresh blood. He collapsed after another hematemesis of about 1000 cc. An immediate transfusion of 500 cc. of whole blood failed to save his life.

At autopsy the prominent findings were in the liver and gastrointestinal tract. There was no ascites. The liver weighed 1705 gm. The surface was nodular and throughout the organ masses of liver tissue from 0.1 to 1 cm. in diameter were separated by fibrous tissue strands. The tissue was of a yellowish brown color. The stomach contained about a liter of bloody liquid while the lower part of the small intestine contained blackish tarry material. Numerous dilated veins were present in the mucosa of the lower part of the esophagus. No actual bleeding points could be found. Microscopically the liver showed a well advanced portal cirrhosis with very little fatty infiltration (Fig. 2).

I have included these cases here to emphasize two things particularly; how, in man, the decreased oxidation of liver fat associated with diabetes results in a chronic fatty infiltration, and how this prolonged infiltration may lead to a cirrhosis of the liver that is indistinguishable from alcoholic cirrhosis. If poisoning by alcohol is added to a preexisting diabetes, the physiological effect should be augmented, as the two conditions, alcoholism and diabetes, are synergistic chemical states.

The Liver in Chronic Alcoholism

In making the following case reports I have attempted to separate those livers in which there was definite cirrhosis from those in which no fibrosis was present, though all of them, except when sudden death occurred, were clinical cases of cirrhosis. This was not easy to do because the fatty liver grades so gradually into the fatty liver with cirrhosis and then into the cirrhosis without fatty infiltration that the dividing lines are by no means distinct. They are based on a study of 47 cases coming to autopsy in which anatomical diagnosis of cirrhotic or alcoholic liver, or both, had been made. Of these cases 3 proved to be toxic cirrhosis, 1 a hepatoma in which a preceding cirrhosis was obvious, and 1 a biliary cirrhosis. The study then includes 42 cases showing the various stages of the lesions to be described. Many others were reviewed but these 42 seemed to me to be conclusive. I have chosen only a few examples for detailed description and these will represent a composite picture. Naturally details were lacking in many clinical histories, particularly the personal details as to diet and the amount of alcohol consumed, so necessary to such a study as this. Also, it is necessary to review these cases backward, from autopsy to history, in order to separate the cases of fatty infiltration only from those of cirrhosis.

Fatty Infiltration of the Liver without Cirrhosis

The typical fatty liver as seen at autopsy in chronic alcoholism has been described. It seems necessary, however, to repeat this with emphasis on certain features that are important in producing the consequent changes leading to cirrhosis. In this series the weight of the liver varied from 1700 to 3600 gm. The enlarged liver has a tense capsule because of definite parenchymal swelling. If the swelling has been chronic or has occurred many times, the capsule is thickened and less transparent than normal. The surface may be coarsely granular or lobulated, even in the absence of cirrhosis. The enlargement and distention of lobules between unyielding interlobular septums which penetrate the liver from the capsule produce an irregular surface that may be accepted as meaning cirrhosis. This lobulation is especially marked during life when the liver is full of blood. The color is usually pale or yellowish red, depending on the amount of blood present. The lobules

may be bile tinged. Fat is frequently present in sufficient amount to grease the knife or the fingers. The consistence is variable but more likely to be firm and tense.

Microscopically these livers show a marked fatty infiltration estimated in some instances as over 60 per cent of the total weight. The fat occurs in typical large globules, and when present in moderate amounts is most often central in location, though this varies in different lobules and seems not to be important. Liver cells at the periphery of lobules may be flattened out, show early atrophy, or may have small amounts of hyaline cytoplasm. Glycogen is usually absent but some may be present when sudden death has occurred and where fat had not completely replaced it. An important finding in many livers is the reproduction of the liver lobules as lobules. In the human liver the lobule may be visualized by noting the spacing of the portal areas usually equidistant from the central efferent vein. From portal area to portal area there is no line of demarcation between adjoining lobules. These become apparent in the excessively fatty liver. Each lobule now becomes a unit and becomes distinctly separable from the other by adjacent rows of compact, squeezed atrophying cells. The superficial appearance again is of cirrhosis, but close examination reveals no fibrous tissue retaining wall as yet, and special connective tissue stains prove the absence of such tissue (Fig. 4).

In most of these livers there is more or less bile retention. This may appear occasionally as fairly large plugs in bile canaliculi, but is most often seen as very small dots of greenish inspissated bile. It may collect in fine curved lines, appearing as incomplete, greenish scaly capsules around fat droplets within a liver cell. This whole picture is that of biliary obstruction, but in no case has there been an extrahepatic block. The obstruction is obviously intrahepatic and is due to the intense and fairly rapid swelling of the parenchyma. This swelling is also responsible for the ascites which is frequently present if the patient has not died suddenly from acute liver insufficiency.

The clinical history and findings in this group differ only in minor details. All individuals had been drinking a great deal for the preceding several weeks or months and had neglected to eat. All had been chronic alcoholics for several years before this last severe episode, and some had been hospitalized before, suffering

from the same symptoms as were present in their last illness. A few examples follow:

CASE 3. An unemployed male, 49 years of age, had been intoxicated for 5 months, during which period his meals were very irregular and often missed. On entry to the hospital he was markedly wasted and had tremor of the arms and legs. He had been a cocaine addict but had not used the drug (so he said) for several years. He died a week after admission with fatty infiltration of the liver. Glucose and a high carbohydrate diet had been given as part of the treatment, which had probably reduced the fat content somewhat.

CASE 4. A female, 47 years of age, who had been a recluse and was known as a chronic alcoholic, increased her alcohol consumption. Three months before entry vomiting spells began, the abdomen became distended, and slight jaundice appeared. In the hospital the liver was found to be large, and ascites and slight jaundice were present. The liver weighed 1550 gm. at autopsy, was markedly infiltrated with fat and contained small plugs of retained bile, but there was almost no interlobular fibrosis.

CASE 5. A male, 37 years of age, died from a fall while drunk. His liver weighed 2800 gm., most of which seemed to be fat. Fat was also present in small vacuoles, resembling an actual fatty degeneration and appearing somewhat similar to the liver in phosphorus poisoning. The only past history known was that he had been a chronic alcoholic.

CASE 6. A male, 40 years of age, entered the hospital with jaundice, nausea and vomiting. He had been on a prolonged alcoholic diet with very little solid food. The jaundice was increasing and the liver enlarging when he was operated upon. A large, swollen nodular liver was seen. Finding no biliary obstruction, an omentopexy was done. A biopsy of the liver showed marked fatty infiltration, outlining of lobules and an increase of fine fibrous tissue connecting portal areas (Fig. 3). The patient recovered and remained entirely well 2 years after complete clinical recovery. It is probable that the change of diet had more to do with recovery than the operation.

These cases present samples of histories that become distressingly monotonous and do not differ qualitatively from those in which a varying degree of cirrhosis was found. The individuals who lived long enough after complete prostration to be studied presented clinical replicas of cirrhosis of the liver. Also, they showed at autopsy few or no other effects of alcoholism such as cerebral edema. Most died in a very few days, sometimes hours, after admission to the hospital. In some instances the liver con-

tinued to enlarge after all alcohol had been stopped, and the jaundice and ascites increased.

LeCount and Singer [40] in 1926 described a similar series of cases. Others are found in French reports. A few citations will represent the surprisingly meager literature. In 1878 Bazy [41] reported a case of a female, 38 years old, who was said to have been intoxicated all the time for an unknown number of years. She entered the hospital with tremors of the hands and face and with a slight jaundice. The liver was large and continued to enlarge over a period of a week until it reached the umbilicus. Jaundice increased progressively. The liver weighed 3520 gm., was of a yellow ochre color and granular on the surface. The lobules were distinct and the capsule fibrosed. There was marked fatty infiltration with early portal cirrhosis. He called it acute yellow hypertrophy.

LeCount and Singer [40] described the large fatty livers found in alcoholics who died rather unexpectedly without delirium tremens or the common findings of acute alcoholism. They noted the lack of glycogen in the liver. They wondered if "whiskey fits" could be due to hypoglycemia, discussed the availability of oxygen for burning both alcohol and fat, and questioned whether the lessened destruction of fat could not be due to a lowered supply of carbohydrate to metabolize fats. So far as cause of death was concerned, the condition of the liver amounted to a virtual extirpation of the organ. Friedewald [42] found that in 7 of 9 autopsied cases out of 50 acute alcoholic deaths enlarged fatty livers were present, and stated that most of the 50 were in a state of starvation.

That fat may infiltrate into the liver so rapidly and to such an extent that intrahepatic block and hepatic insufficiency ensue is shown by the occasional occurrence of the same phenomenon as recorded here in depancreatized dogs. They have died with jaundice and a swollen fatty liver, but without evidence that the diabetes, beyond being responsible for the fat, was very severe.

Allan, Bowie, Macleod and Robinson [37] perhaps first described this phenomenon. Three depancreatized dogs died and jaundice and bile-stained fatty livers were present. There were some degenerative changes in liver cells around the central veins, marked fatty infiltration, and the bile canaliculi contained bile. Fisher [43] described essentially the same findings in two dogs. In the course of experiments on depancreatized dogs it was found that this con-

dition was precipitated when strict dieting and insulin dosage were not rigidly controlled.* Four such cases occurred in the large group of dogs used to produce the cirrhosis of the liver referred to above. These four dogs became emaciated, jaundiced, did not eat, and died in from 20 to 40 weeks after the pancreas had been removed. At autopsy the livers were markedly enlarged, filled with fat, and there was marked bile retention. There was no extrahepatic block and nothing else to account for jaundice except intrahepatic swelling. Pictures of them could be substituted for those shown here from the livers in chronic alcoholism.

If I have somewhat belabored this phase of a subject, which should be common knowledge to clinicians and pathologists, it has been with the deliberate intent to emphasize that a pathological condition exists which is not cirrhosis, but because of the history of alcoholism, the ascites, moderate jaundice and frequent emaciation, the clinical condition of cirrhosis of the liver may be duplicated. A continuation of this condition or reproduction of the condition so frequently that in between times the liver has not had time to return to normal, will lead to the irreversible histological changes of cirrhosis.

Alcoholic Cirrhosis Following Prolonged Fatty Infiltration of the Liver

The clinical histories in most cases of definite cirrhosis, early or late, may, I think, be omitted. These individuals all have several common factors: they have consumed a great deal of alcohol during the last few years before death; they have been (when definite inquiry was made) on a very irregular diet, sometimes amounting to complete fasting, and nearly always low in carbohydrates; they have usually become wasted, or have lost considerable weight; and the age is usually above 45 years. I shall record the following case only because of the authentic history and the clear-cut sequence of events, both clinically and pathologically.

Case 7. A female, 35 years old, who had been of temperate habits, began to drink heavily following a family catastrophe. At the end of 23 months she deliberately set out to drink herself to death and achieved this end in another 30 days. During the 2 years she had eaten very irregularly, and during the last month almost nothing.

* Experiments in collaboration with Dr. I. L. Chaikoff.

At autopsy the liver weighed 1800 gm., was of a yellow color, and had the characteristic lobulated surface of cirrhosis. On section the lobules were distinct, all of them larger than normal but irregular in size and shape.

Microscopically fatty infiltration was so extensive that nearly every cell contained a large or small globule. The sinusoids and central veins were collapsed, invisible, and a well defined fibrous tissue wall was growing around the lobules and sometimes through them (Fig. 6). Close examination revealed the changes that are so frequently found in this group of cases when the degeneration and fibrosis are rapidly progressing; changes that must be considered pathognomonic of developing fatty cirrhosis. These are: atrophy of liver cells at the periphery of lobules (Fig. 7); hyaline degeneration of swollen cells, many containing rounded or irregular masses of hyaline cytoplasm (Fig. 8); the development of thick reticulum fibrils around degenerating cells and along the course of sinusoidal walls (Fig. 9); and condensation of reticulum and further fibroblastic proliferation to form the well defined, perilobular connective tissue which is definitive.

Because most cases occur during the 5th and 6th decades, the statement is usually made that it requires many years for alcoholic cirrhosis to develop. This, as we have seen, is not true. The fact is that men do not begin to drink the astonishing amounts needed to cause cirrhosis until, as a rule, after the age of 45 years. The amount consumed previous to this period has been of no importance if it has been moderate. Varying the degree of alcohol poisoning, and variations in food consumption may prolong the process indefinitely. The maintenance of physiological metabolic equilibrium within safe limits may be possible even when comparatively large amounts of alcohol are taken habitually.

The Development of the Lesion of Cirrhosis in Fatty Livers

There seems to be no room for doubt but that long continued fatty infiltration of the liver is a mechanical factor of great importance in the development of a fibrous tissue-retaining wall around distended lobules. This had caused collapse of sinusoids throughout the liver. In addition to pressure on the peripheral cells, there is a definite reduction in circulation and consequent anoxemia. Slow atrophy of cells is the usual result. Other livers

undergo a hyaline degeneration, sometimes with clumps of hyaline cytoplasm appearing in cells, reproducing the picture described by Mallory years ago (Figs. 7 and 8). In more acute cases there may be an associated coagulative and fatty necrosis of cells, though this is not so common and not essential. This type of degeneration is seldom so severe as when found in chloroform, carbon tetrachloride and phosphorus poisoning. It is most commonly found in instances of acute death. The liver that develops outstanding cirrhosis must survive such episodes.

Connective tissue proliferation begins anywhere in the periphery of the lobules from the cells forming the walls of sinusoids. It is frequently more prominent around the portal areas but it does not necessarily grow out from them. There is usually a thickening of the sinusoidal wall as the first noticeable feature. The normally thin wall of scarcely recognizable collagen fibrils becomes swollen, forming homogeneous thicker lines of collagenous material, and a delicate reticulum can be seen to form around degenerating cells. The fibroblastic proliferation that follows is a familiar phenomenon and the fibrous strands thus formed connect collapsed empty capillaries (sinusoids) with one another, eventually joining with prolongations from periportal connective tissue. In the average slowly developing case these strands more or less clearly define the limits of a lobule, with an efferent vein in the center and the now united portal areas forming "fence-posts" at regular intervals. These strands, however, may join with others coming from any direction and so cut across lobules, forming the irregular lobulated pattern that has been so hard to explain. The process after this is simply the development of more and more connective tissue with subsequent condensation and some attempt at regeneration of bile ducts and liver lobules (Fig. 10).

The disappearance of fat from the liver in advanced cirrhosis is readily explained, although the explanation in each case may not always be the same, or a combination of factors may operate in the same case. In the experiments previously described concerning depancreatized dogs, fat accumulation in the liver remained for periods varying from 3.5 to 4.5 years. These diabetic dogs were receiving insulin and a diet that allowed them to live comfortably without marked metabolic imbalance. In animals allowed to live for several years, varying in each animal, fat gradually disappeared

from the liver and cirrhosis began to be noticeable. Among eight dogs there were four with fatty infiltration and cirrhosis, and four with cirrhosis and little or no fat. Kaplan and Chaikoff [44] have shown elsewhere that fat diminishes from a high of 40 to 50 per cent in the course of time in depancreatized dogs and the animals become emaciated.

Dible,[45] and Dible and Libman [46] have shown that the degree of fatty infiltration of the liver during fasting (mice, rats, rabbits, guinea pigs) depends on the amount of fat present in the body. When the fat depots are depleted fatty infiltration does not take place. One reason then for the disappearance of fat in fatty cirrhosis is exhaustion of the fat reserve of the body. Another and perhaps more important reason is that alcohol consumption becomes less and cautious dieting begins when the cirrhosis has developed to the extent that a gradual chronic passive congestion of the gastro-intestinal tract becomes manifest. With the resumption of a normal diet, particularly as carbohydrates are increased, fat rapidly disappears from the liver. It is to be expected then that in patients who have been hospitalized for some time the liver will become devoid of fat. Especially is this true latterly because of the empirical use of glucose solution in all cases in which a damaged liver is suspected. Frequently, then, all signs of fatty infiltration — cell atrophy, hyaline degeneration, and even active fibroblastic proliferation — have disappeared and we see the familiar quiescent, fibrotic, now somewhat shrunken, nodular liver of alcoholic cirrhosis.

Summary and Conclusions

Fatty infiltration of the liver occurs in those conditions where, because of lack of intake or absorption of food, fat is mobilized from the existing fat depots; and where, because of internal interference with the metabolism of fat due to anoxemia or tissue anoxia, the accumulated fat cannot be broken down for use. In the first instance it results from external starvation; in the second from what may be called internal or tissue starvation. In both instances normal carbohydrate-fat metabolism does not take place. Among the conditions in which this normal metabolism is altered or inhibited are the various diseases which, by their nature, are called wasting; the disease diabetes; and that following the intro-

duction of poisons which inhibit proper tissue oxidation, the most common of which is alcohol. Others occur but are not dealt with here.

In the starvation accompanying progressive morbid states the condition is of little pathological importance, being in most cases terminal in nature. In diabetes the enlarged fatty liver may be influenced by insulin and by strict dieting, but also may persist for many years. In alcoholism a variety of factors operate to produce the same condition. Alcohol alone will cause some fatty infiltration, but as relative and sometimes absolute starvation is constantly associated with severe chronic alcoholism, the development of fatty infiltration of the liver most often depends on a combination of these two. The absence of vitamin B_1 in the diet may contribute to this also, but such deficiency is probably of minor importance.

Experiments recited here, and an analysis of existing recorded observations and experiments, indicate that a liver containing demonstrable neutral fat is in most cases pathological; that fat may pass into and out of the liver with astonishing rapidity; and that fat may be present in such amounts as to interfere seriously with both the metabolic and the mechanical functions of the organ. In 2 cases of diabetes this chronic fatty infiltration went on to portal cirrhosis. One of these cases was so severe that the patient died of hemorrhage from esophageal varices. The development of perilobular fibrosis seems to be the result of a combination of mechanical pressure, local tissue anoxia and general anoxemia, causing atrophy and degeneration of liver cells.

The alcoholic liver occurs in two forms, one of which is the precursor of the other. The first is the enlarged tense liver so swollen with fat that the distended surface lobules present the appearance of cirrhosis. This effect is further simulated by the intrahepatic block, which interferes with excretion of bile, and the transmission of portal blood. The clinical signs of jaundice and ascites are thus produced. Many such patients lapse into coma and die, and at autopsy the large liver is the only prominent finding. These show lobules distended with fat and some show, in addition, hyaline degeneration and atrophy of peripheral cells associated with early proliferation of fibroblasts. A series of cases presented here represents this group, and another series illustrates

the second form in which further proliferation of connective tissue produces the typical picture of portal cirrhosis as seen in chronic alcoholism. An unmistakable gradation of the one into the other is so manifest that the mechanism of the production of alcoholic cirrhosis seems to me to have been demonstrated. The absence of fat in many such livers at the end is explained by the exhaustion of body fat, by the discontinuance of alcohol consumption, and the resumption of a normal or a high carbohydrate diet.

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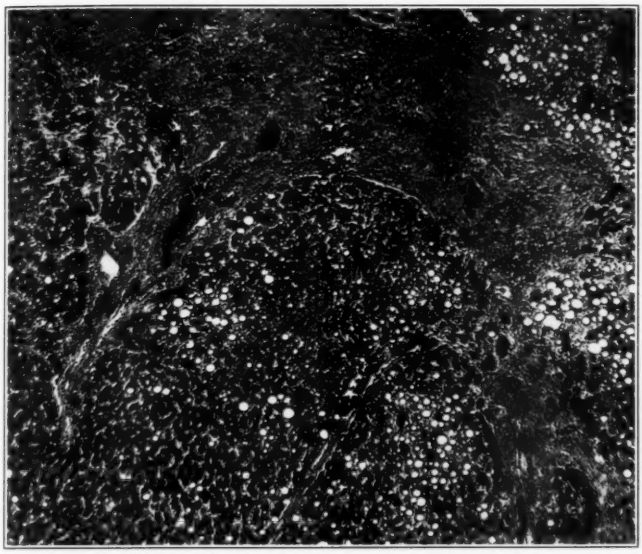
DESCRIPTION OF PLATES

PLATE 69

FIG. 1. Microphotograph of liver showing fatty infiltration, perilobular and intralobular fibrosis in a case (Case 1) of diabetes of 17 years duration. Death from diabetes. Hematoxylin-eosin stain. × 50.

FIG. 2. Microphotograph of liver (Case 2) showing perilobular fibrosis (portal cirrhosis) in a liver which was known to have been enlarged (presumably fatty) 8 months previously. Death from portal obstruction, esophageal hemorrhage. Hematoxylin-eosin stain. × 50.



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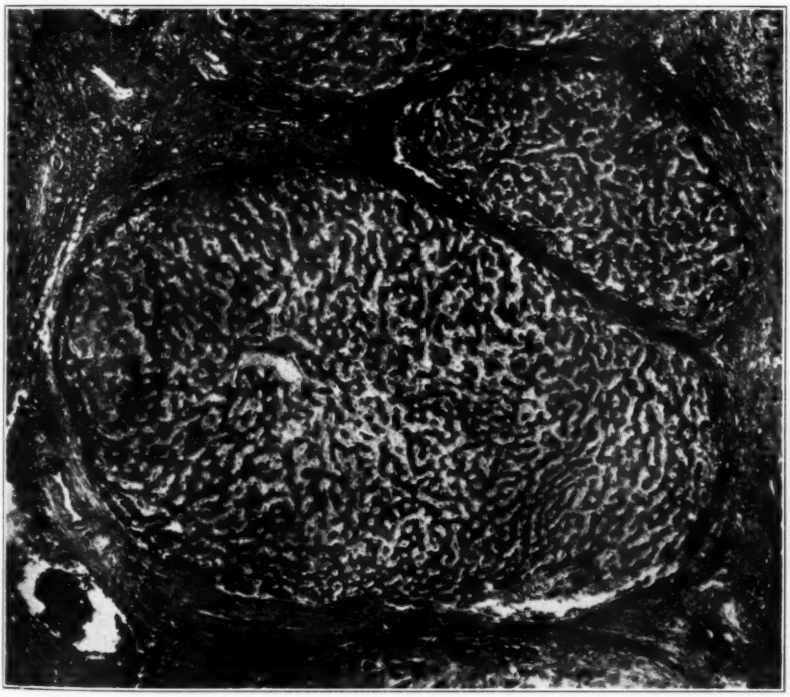

2

Connor Fatty Infiltration of Liver

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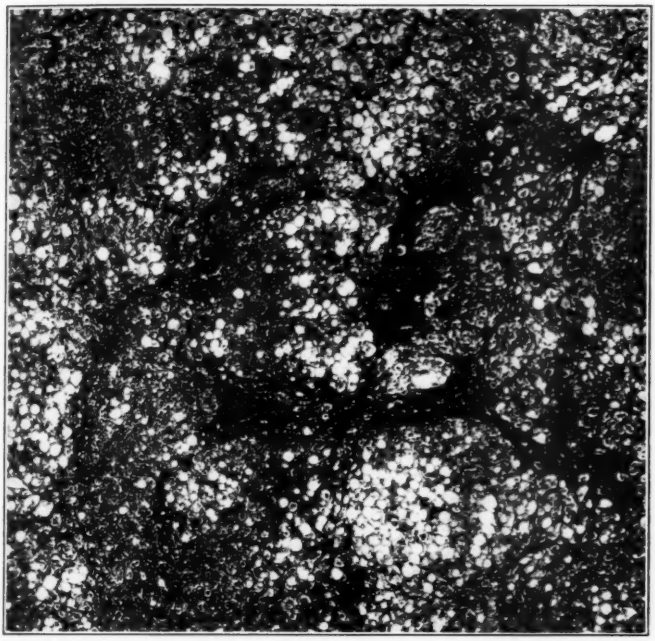
PLATE 70

FIG. 3. Microphotograph of liver. A biopsy specimen from a patient who recovered and has remained well. The effects of alcohol are seen in the marked fatty infiltration, peripheral cell degeneration and connective tissue proliferation. A borderline change which has returned to a normal physiological state so far as can be determined, but which, it seems obvious, could progress into the irreversible condition of cirrhosis. Mallory's aniline blue collagen stain. × 60.

FIG. 4. Microphotograph of liver showing fatty infiltration (acute) from a male who died of alcoholism. The outlining of the lobule is evident. This represents the physiological lobule in man, which anatomically is not so distinct as in some lower animals. This could not now be called cirrhosis but the foreshadow is obvious. Hematoxylin-eosin stain. × 60.



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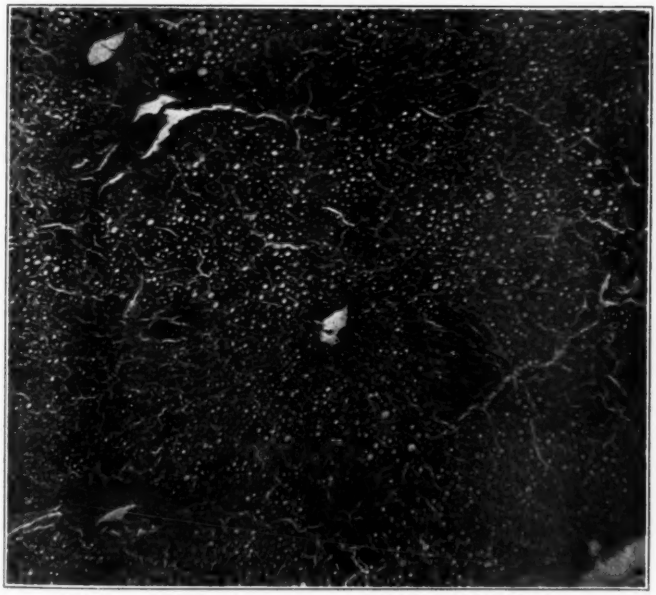

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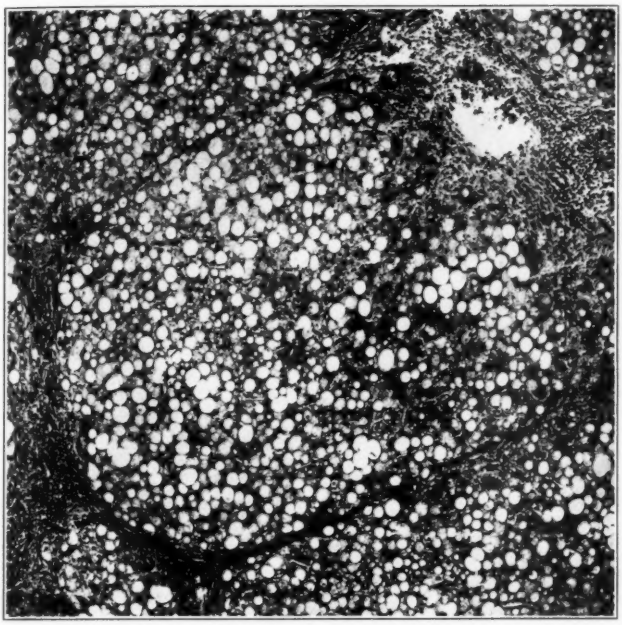
PLATE 71

FIG. 5. Microphotograph showing the beginning of cirrhosis in a fatty alcoholic liver. A wall of fibrous tissue now surrounds the lobule forming an anatomical as well as a physiological unit. Hematoxylin-eosin stain. × 60.

FIG. 6. Microphotograph of liver showing early but definite cirrhosis of the liver of the rapidly progressing alcoholic type. This section contains all the cellular and tissue changes which characterize the histogenesis of alcoholic cirrhosis. Modification of Masson's trichrome stain. × 60. These are shown in Figs. 7, 8 and 9. Figs. 6, 7, 8 and 9 are from Case 7.



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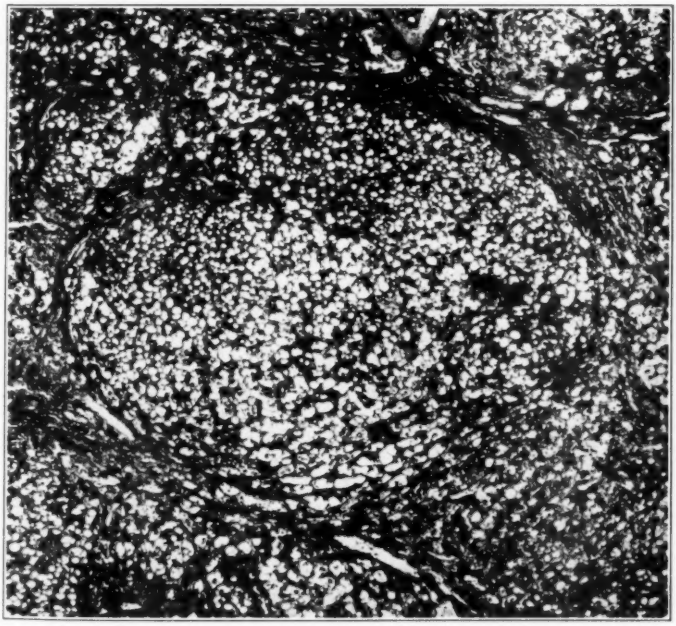

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Connor Fatty Infiltration of Liver

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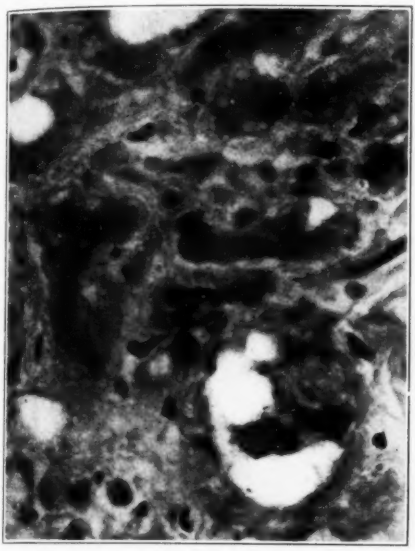
PLATE 72

FIG. 7. Microphotograph of liver showing atrophy and degeneration of cells at the periphery of a lobule in "acute" cirrhosis. Hematoxylin-eosin stain. × 500.

FIG. 8. Microphotograph showing the hyaline clumps described by Mallory, which are present in progressive cirrhosis but absent in the quiescent phases. Fatty infiltration, fatty degeneration and cell atrophy can be seen also. Proliferating stroma not easily recognized by this technique. Picture taken with green color filter to bring out hyaline masses in cells undergoing degeneration. Hematoxylin-eosin stain. × 500.

FIG. 9. Microphotograph of liver showing the development of reticular and collagen fibrils around a lobule and between cells, following the natural line of cleavage along a bloodless capillary. Masson's trichrome-silver method. × 600.

FIG. 10. Microphotograph of liver showing well advanced cirrhosis with some residual fat and clumps of atrophic cells persisting in the fibrous tissue. From a seaman, 50 years of age, who drank a great deal of whiskey at intervals, and some all the time. Three years before death he had been in the same hospital with vomiting, jaundice and an enlarged liver. On his last admission there was no jaundice, the liver was smaller, and he presented the typical findings clinically and at autopsy of cirrhosis of the liver. Hematoxylin-eosin stain. × 50.



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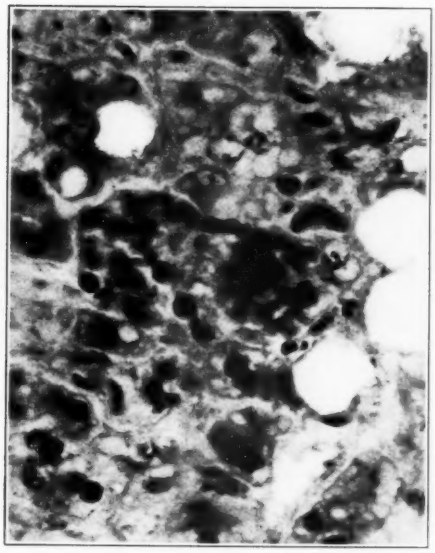

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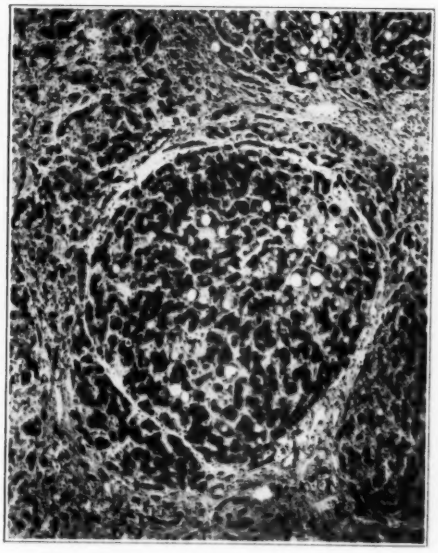

10

Connor

Fatty Infiltration of Liver

PRIMARY RETICULUM CELL SARCOMA OF THE LYMPH NODES OF A COW WITH WIDESPREAD METASTASES *

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INTRODUCTION

Conditions resembling lymphoid hyperplasia are frequently observed in food-producing animals and are usually thought to be a leukemia or leukemic-like disease arising from the lymphoid elements or the cells of the lymphocytic series. European investigators have from time to time made studies of leukemia and leukemic-like diseases in such animals, but it appears that comparatively little investigation has been done along this line in the United States. A review of the literature presents a bewildering discussion dealing with lymphatic leukemia, pseudoleukemia, lymphadenoma, leukemia, lymphoblastoma, lymphocytoma, lymphosarcoma, alveolar sarcoma and round-cell sarcoma. It appears that the various authors in describing cases belonging to this group of diseases have selected from these terms those which they thought most appropriate for their particular cases without making any detailed or intensive study of the underlying histopathological changes. Furthermore, the opportunity for making such detailed studies on the living animal has been rather infrequent because this group of diseases is rarely detected in the live animal and is generally first seen after the animal has been slaughtered for food purposes. In emaciated animals it may sometimes be possible to recognize these so-called lymphoid hyperplasias due to the prominence of greatly enlarged superficial lymph nodes, but the vast majority of these conditions are not detected or even suspected in the various food animals until after they are slaughtered and a postmortem examination made.

In Germany Ostertag [1] has written considerably concerning these lymphoid hyperplasias or so-called leukemias in food-producing animals. He considers lymphoma, on account of its frequency and importance in meat inspection, to be the most

* Received for publication December 29, 1937.

important tumor of lymph glands and states that it occurs in connection with lymphatic leukemia. A characteristic feature of lymphomas is their simultaneous appearance in numerous lymphatic glands as well as in the lymph follicles of various organs. At the same time diffuse leukemic infiltration may occur in organs such as the liver and kidneys which do not belong to the lymphatic system.

Pseudoleukemia, in which anatomical changes similar to those of true leukemia occur in the spleen, bone marrow, lymphatic glands and other parts of the body without any change in the numerical ratio of the white or red corpuscles, is much more common than true leukemia in food-producing animals, according to most investigators. The term lymphogranulomatosis or false pseudoleukemia has been applied to Hodgkin's disease or Sternberg's pseudoleukemia as in this affection the nodules in the lymphatic glands, spleen, liver, kidneys and lungs consist of typical granulation tissue formed of plasma cells, lymphocytes, leukocytes and Sternberg's giant cells. On the evidence of this histological picture, the occurrence of Hodgkin's disease in food animals has not been proved, but MacMahon,[2] and Stalker, Schlotthauer and Feldman[3] have encountered this disease in dogs, while Medlar and Sasano[4] reported a neoplasm in a rabbit which corresponded closely to Hodgkin's disease in man.

Lymphosarcoma is thought to be common in animals and bears a gross resemblance to leukemia. It is described as beginning with tumor formations in the lymphatic glands, leads to infiltration of the surrounding tissue, and may give rise to numerous lymphatic nodules by way of the lymph stream, and also form metastatic deposits in the spleen, liver and bone marrow. Therefore, it is said to occupy an intermediate position between pseudoleukemia and malignant tumors. Lymphosarcoma of dogs, which is transmissible from animal to animal, is thought to be a true tumor formation, manifested by multiple tumors formed of small round cells resembling lymphocytes. It is considered by some investigators to be transmitted by a filterable virus.

Many comparative pathologists believe that the primary types of progressive lymphoid hyperplasia are true neoplasms and consider them so closely related genetically as to justify their classification in one group known as lymphoblastoma, as suggested by

Mallory.[5] In the Federal Meat Inspection Service these malignant and metastatic lymphoid hyperplasias are usually grouped under the collective term "pseudoleukemia." All species and ages of food-producing animals are found to be affected. Condemnations for pseudoleukemia, as published in the report of the Chief of the Bureau of Animal Industry of the United States Department of Agriculture, for the fiscal year ending June 30, 1936, are given in Table I.

TABLE I

Comparison of the Number of Food-Producing Animals Condemned for Pseudoleukemia with the Total Number of Animals Condemned on Postmortem Examination by the Federal Meat Inspection Service of the Bureau of Animal Industry During the Fiscal Year Ended June 30, 1936

Species	Total number examined	Total number condemned	Per cent condemned	Condemned for pseudoleukemia	Per cent condemned for pseudoleukemia
Cattle	10,298,213	69,595	0.67	1385	0.0134
Calves *	5,783,154	19,340	0.33	50	0.001
Sheep	17,316,665	36,028	0.20	20	0.000115
Goats	51,464	185	0.36	1	0.07
Swine	28,506,019	86,820	0.30	180	0.00063
Horses	14,899	136	0.90	0	0.0

* Aged 1 year or less.

Undoubtedly cases of true leukemia do occur among food-producing animals and a number of cases thought to be true leukemia have been reported in the literature, but many of them are open to question because of insufficient cytological study. Creech and Bunyea [6] studied a case in a cow in which the definite changes in the cell ratio proved to be a true leukemia, the white cell count reaching a level of 203,250. The case reported by Gray [7] as lymphatic leukemia in a 5 year old Jersey cow lacks convincing proof that it actually was a true leukemia. Feldman [8] prefers to place the conditions known in veterinary literature as leukemia, pseudoleukemia, malignant lymphadenoma, lymphosarcoma, and many others, under the designation of lymphocytoma. Experienced veterinary pathologists admit that it is difficult to distinguish between leukemia, pseudoleukemia, and allied conditions, either macroscopically or microscopically. Some investigators consider the presence of an excessive number of white blood cells in the blood remaining in the vessels of cut and stained sections of

various tissues and organs affected with lymphoid hyperplasia sufficient evidence to distinguish between leukemic and aleukemic conditions. I doubt the wisdom of such a practice as I do not think the condition of the blood in the vessels of cut and stained sections of tissues affords definite evidence of the condition of the circulating blood in the live animal.

I have studied many cases of these so-called leukemias, or lymphoid hyperplasias, in the different species of food-producing animals with a view of obtaining more definite knowledge concerning them. Various obstacles have interfered with a complete detailed study of these cases. In the first place opportunity was not afforded for making a clinical examination of the live animal with blood studies, (2) some other person usually performed the postmortem examination and selected the tissues for study, and (3) the tissues submitted for examination were frequently not fresh enough for a detailed cytological study.

A supervising inspector recently called our attention to a cow that had a number of the superficial lymph glands greatly enlarged. This cow was one of a lot of cattle received at the Chicago stockyards for slaughter. After clinical and histopathological studies of the case the conclusion was reached that the animal was affected with a primary reticulum cell sarcoma of the lymph glands with widespread metastases. Because such cases have not previously been reported in veterinary literature (so far as I am aware) the case is considered of sufficient interest and importance to warrant reporting in detail.

Report of Case

Clinical Findings and Blood Examination: The subject was a large Holstein cow approximately 8 years of age and in good flesh. The animal walked with an even steady gait but the prescapular and precrural lymph nodes stood out very prominently. The left prescapular lymph node appeared to be about 18 by 26 cm., while the right prescapular lymph node and both precrural lymph nodes appeared to be about 7 cm. in diameter. No other enlarged lymph nodes could be detected on palpation. A number of blood smears were prepared from blood drawn from an ear vein and stained with Wright's and Giemsa's blood stains. On microscopic examination these blood smears were normal. No abnormal erythrocytes were

observed and there was not an excessive number of leukocytes present.

The cow was bled again 2 days later for the purpose of making further blood counts. A test for hemoglobin was also made at this time, using the Tallqvist hemoglobin scale, and a reading of about 65 was obtained. Blood counts made from the oxalated blood from the jugular vein revealed about 6,000,000 erythrocytes and 6900 leukocytes per cmm. with 31 per cent polymorphonuclears, 51 per cent lymphocytes, 9 per cent monocytes and 9 per cent eosinophiles. The cow was slaughtered and autopsied on the following day.

Postmortem Findings

The carcass was that of a fairly well nourished mature cow with a normal fat content and distribution for this particular type of animal.

Head: The mandibular, parotid, atlantal and retropharyngeal lymph glands were moderately enlarged and grayish white in color. They were somewhat softer in consistence than normal.

Lungs: The lungs and associated lymph glands were essentially normal in appearance and consistence. The parietal and visceral pleura were smooth and glistening. In the anterior portion of the thoracic cavity on the left side was a mass of tissue measuring 10 by 6 cm. This mass of tissue was grayish white and moderately soft, but contained no recognizable thymic tissue.

Heart: The emptied heart weighed 4300 gm., which is about normal for an animal of this size. The myocardium was firm but contained a large amount of a grayish white tissue, which extended through the entire wall of the myocardium. Both auricles were almost completely replaced with a similar grayish white, moderately firm tissue.

Liver: The liver was normal in size, consistence and color.

Spleen: The spleen was normal in size but was firmer than usual. The capsule was thickened and fibrous tags adhered to it. The splenic pulp was normal in appearance.

Kidneys: Both kidneys were enlarged, each measuring 22 by 14 by 8 cm. Extending above the surface of both kidneys were a number of large, light colored nodules which upon being incised were found to contain copious amounts of a yellow semifluid pus.

Only a thin shell of kidney tissue remained at the periphery. This condition was diagnosed as bilateral pyonephrosis.

Intestines: The small intestines contained throughout their entirety innumerable nodules or deposits in their walls, which were more noticeable as a rule on the serous than on the mucous surface, although many nodules were distinctly visible on the mucous surface and protruded for varying distances into the lumen of the intestines. These nodules measured up to 6 cm. in diameter and were grayish white, moderately firm and had a more or less irregular surface contour. The whole chain of mesenteric lymph glands was markedly increased in size, grayish white and moderately firm. On incision they appeared moist. Practically all the visceral lymph glands in the abdominal cavity were enlarged and resembled the mesenteric lymph glands in color and consistence.

Uterus: The uterus contained what was approximately a 5 months fetus. The whole uterine wall was greatly thickened, measuring as much as 3.5 cm. in some places. This thickened uterus was very pale in color, almost a grayish white and was moderately firm. Both ovaries appeared normal. The various internal organs and lymphatic glands of the fetus showed no visible abnormalities.

Skeletal Muscle: In the muscles of the flank on the right side a grayish white area measuring 16.5 by 7.5 cm. was noted. This grayish white area replaced the muscles in the area involved and was moderately firm.

Lymph Glands: The left prescapular lymph gland was enormous in size and adherent to the surrounding tissues. It measured 43 by 32 by 20 cm. Sisson [9] gives the normal dimensions of this gland as 10 to 12 cm. long and 3 cm. wide. The gland was grayish white with a slight mottling of red and contained a few small necrotic areas. It was moderately firm and the cut surface appeared somewhat moist. The right prescapular lymph gland was also enlarged, measuring 24 by 12 by 10 cm., and resembled the left prescapular lymph gland in appearance and consistence. The left precrural lymph gland measured 28 by 14 by 13 cm., and the right precrural lymph gland measured 18 by 8 by 6 cm. Sisson [9] gives the normal dimensions for these glands as 8 to 10 cm. long and 2.5 cm. wide. The color and consistence of these glands were similar to those of the prescapular lymph glands.

Bone Marrow: The bone marrow in the medullary cavities in the diaphyses of the tibia, femur and humerus was found to be of a normal yellow fatty consistence. The bone marrow of the ribs was deep red in color.

Tentative Anatomical Diagnosis: The lesions noted in this case were considered typically characteristic of the condition in cattle commonly designated pseudoleukemia.

Histopathology

The neoplasm was essentially the same in all sections, consisting of an intermingling of two types of cells. One of these which predominated in all parts of the growths had features of malignancy and was considered the essential cell. It was identified as a malignant reticulum cell. These cells grew in sheets or formed irregular alveoli of cells and infiltrated other tissues freely. They varied in size and in shape and were from 15 to 30 μ, or even more, in diameter. The cytoplasm was moderately abundant, clear, without specific granulations, and slightly oxyphilic in staining. When not altered by pressure relations the cells tended to be oval, although the cytoplasm often streamed off into points. The nuclei occupied the major portion of the cells and tended to be oval, although many were kidney shaped, lobulated or knobbed. The chromatin in the nuclei varied greatly in amount and distribution, but on the whole was very abundant. In some of the cells the chromatin of the nuclei was in the form of coarse granules while in others it was finely distributed. The nuclei tended to be vesicular. Nucleoli were visible when the chromatin was not excessive. They were large, slightly acidophilic, and varied in number from one to three. Mitotic figures were numerous in the various sections, over twenty being visible in some high power fields. They were often abnormal and bizarre in character, showing unequal division of chromatin. Foot's modified silver method for reticulum fibers showed a uniform fine network so that nearly every tumor cell was conceivably in direct contact with a fibril. These tumor cells were phagocytic for other cells and cell débris in places where there was active cell disintegration.

The second type of cells seen in small numbers in all sections was of the erythropoietic series. These cells were polychromatophilic erythroblasts, erythroblasts and normoblasts. An occasional

cell of this type was seen among the tumor cells in all sections. In some blood vessels and lymphatic channels of the lymph nodes they were seen in clumps and groups. This same erythropoiesis was also seen in places where it was not associated with the neoplastic changes, for example in the spleen capsule. It did not appear to be an essential part of the tumor process.

Lymph Glands: The normal architecture was completely destroyed by an overgrowth of tumor cells. Only an occasional sinus and blood vessel was recognizable. The capsule was slightly thickened by dense fibrous connective tissue and was heavily invaded by the anaplastic tumor cells.

Heart: The heart was diffusely invaded by the tumor cells from the epicardium to the endocardium. They did not push the muscle fibers aside but actually destroyed them by invasion. The muscle thus invaded showed various stages of degeneration.

Skeletal Muscle: The same characteristic feature noted in the myocardium was also seen in the skeletal musculature in that the tumor cells actually invaded and destroyed the muscle fibers rather than by spreading the muscle fibers apart. Sheets of tumor cells were present in the invaded muscle. The muscle fibers showed atrophic and degenerative changes.

Intestine: The intestinal nodules were composed of tumor cells of the type already described which had invaded and destroyed the entire muscularis mucosa and mucosa. Only remnants of the glandular and muscle structures remained. Although these nodules were fairly well circumscribed, they could be seen projecting into the surrounding intestinal wall. Adjacent to the nodules the mucosa was heavily infiltrated by lymphocytes, plasma cells and leukocytes, especially eosinophils.

Uterus: The entire wall was to a large extent replaced by tumor tissue as above described and consequently was greatly thickened.

Liver: The normal architecture was well preserved. The periportal areas showed a moderate increase in cellularity, mainly of histiocytic proliferation. The Kupffer cells were numerous but showed no definite phagocytosis. In the sinusoids there was noted an occasional bone marrow giant cell or lymphocyte. Small nests of rather large cells with vesicular nuclei and the chromatin arranged around the periphery of the nuclear capsule were found scattered irregularly throughout the liver. Some of the liver cells

showed hydropic degeneration. Some of the larger blood vessels contained lymphocytes and occasional bone marrow giant cells. Many of the sublobular veins contained large numbers of mononuclear cells and some lymphocytic and monocytic cells. There was no tumor infiltration in the liver.

Spleen: The follicles were well preserved and distinct but showed no definite germinal centers. The pulp showed a marked increase of fibrous tissue. The spleen also showed much golden brown pigment in macrophages, and erythropoiesis in the sinusoids and red pulp. The capsule was thickened by dense fibrous connective tissue. A fibrous tag attached to the capsule was traversed by large sinuses, some of which were filled with red cells, lymphocytes and blood pigment. There was no tumor infiltration in the spleen.

Bone Marrow: The red bone marrow of the rib showed active granulopoiesis and hematopoiesis. There were various stages from erythrogonia to normoblasts and erythrocytes as well as myeloblasts and metamyeloblasts and granulocytes. Bone marrow giant cells were found throughout, some having innumerable lobed nuclei. This was a very active but normal bone marrow.

Final Anatomical Diagnosis: Primary reticulum cell sarcoma of the lymph nodes with metastases in the heart, intestines, uterus and skeletal muscles.

Discussion

No evidence of myelopoiesis was found in any of the tumor sections studied and there seems to have been no tendency for the cells to mature in the direction of myelocytes. Extramedullary erythropoiesis is very common in the spleen, liver, lymph nodes, kidney, and so on, in instances of carcinoma as well as in other chronic diseases, but it is not usually seen in the tumor itself, as in this case.

No proliferation or participation of the vascular endothelium in the tumor process was noted, and all blood vessels apparently resisted invasion by the tumor cells.

Ewing [10] distinguishes between endothelioma of lymph nodes arising from the endothelial lining of lymph and cavernous sinuses, and a loosely arranged, large celled lymphosarcoma arising from the reticulum cells of the lymph nodes. He regards them as spe-

cific diseases based on their clinical course and microscopic picture. Concerning the histogenesis of these tumors Ewing states that the reticulum cells of the lymph nodes are derived from connective tissue cells and form a meshwork in which lymphocytes gather from without. The reticulum of the follicles and pulp does not produce lymphocytes but these two cells constitute separate series. From the reticulum cells of the follicles can be traced the development of large round cell tumors of the type of large round and giant cell lymphosarcoma, which must be separated from lymphocytoma. The tumors composed of loose round cells derived from the reticulum of the follicles differ markedly from the others, and it seems unwise to urge any change in their usual designation as reticulum cell sarcoma.

Ewing does not mention the argyrophilic reticulum, which is a constant feature of tumors of reticuloendothelial origin. In the microscopic diagnosis of reticulum cell sarcoma of the lymph nodes silver impregnation and fat stains are obligatory in order that the characteristic argentophil fibrils and lipoids may be brought out. Based on extensive study of neoplastic conditions Callender,[11] registrar of the American Registry of Pathology, regards the blood monocyte and the reticuloendothelial cell as being one and the same cell, and states that the reticulum cell sarcoma really includes Hodgkin's sarcoma and is a better term for the condition.

"Retotheal sarcoma" is a term suggested to Roulet by Rössle, who used it in articles published in 1930 and 1932 to replace the term "reticulum cell lymphosarcoma." The derivation is from the German "Retothelien" (reticulum). The term is more accurately used, however, as a contraction of the bulky term reticuloendothelial because the reticular element should not be separated from the endothelial element in this system. Reticulum cell lymphosarcoma, or retothal sarcoma, was recognized as a type of lymphosarcoma by Ghon and Roman in 1916. Because the relation of the lymphoblast to the reticulum cell was not then clear, it had been considered as related to lymphoblastic lymphosarcoma, but Roulet clearly demarcated it from the latter.

Sarcomas as a rule do not invade the lymphatics but metastasize by way of the blood stream; but lymphatic and reticulum cell lymphosarcoma, which originate in lymph nodes, may travel by

way of both lymph and blood vessels, so that these tumors may yield the most abundant and widely disseminated metastases seen with any type of neoplasm. The leukemic equivalent of reticulum cell sarcoma, namely a monocytic leukemia, should eventually be discovered in food-producing animals.

Summary and Conclusions

The case of an 8 year old Holstein cow affected with greatly enlarged superficial lymph nodes is presented. Clinical examination of the blood revealed a hemoglobin index of 65 (Tallqvist), and normal white, differential and red cell counts. Postmortem examination revealed widespread enlargement of the lymph nodes with tumor formations, considered to be metastases, in the heart, intestines, uterus and skeletal muscles. The lungs, liver, spleen and bone marrow were not invaded by tumor growth. Microscopically the enlarged lymph nodes and tumor growths in the heart, intestines, uterus and skeletal muscles were identical, being composed principally of large, irregularly shaped cells measuring up to 30 μ or more and containing large, oval, kidney shaped, lobulated or knobbed nuclei which tended to be vesicular. Mitotic figures were exceptionally numerous, twenty or more being visible in some high power fields. Silver impregnation revealed a uniform fine network of argyrophil fibers virtually in direct contact with every tumor cell. Many of the tumor cells were phagocytic for other cells and cell débris. Scattered throughout the tumor cells were small areas of erythropoiesis which did not appear to be an essential part of the tumor process. The tumor cells proper in this case are believed to be malignant reticulum cells having their origin in the reticulum of the lymph nodes. These reticulum cells are not lymphocytes but are of connective tissue origin, and tumors originating from such reticulum cells constitute a specific group that must be separated from lymphocytoma, pseudoleukemia and lymphatic lymphosarcoma.

Although reticulum cell sarcoma has not been reported previously in food-producing animals, I do not believe this is a rare neoplasm. When fresh tissues are obtained and sections are appropriately stained and the silver impregnation method used, it is believed that many cases of reticulum cell sarcoma will be diagnosed in animals which were formerly considered to be disturb-

ances of the lymphoid elements and diagnosed as leukemia, pseudoleukemia, lymphosarcoma, lymphocytoma, and so on.

Note: I wish to express my appreciation to Dr. S. R. Rosenthal and to Dr. P. E. Steiner for the valuable advice and information which they so cheerfully furnished while making these studies.

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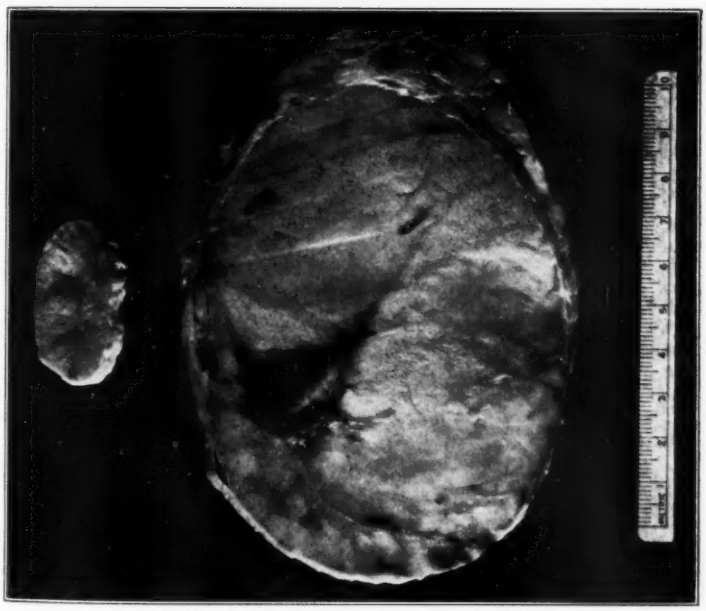
DESCRIPTION OF PLATES

PLATE 73

FIG. 1. Cross section of precrural lymph node of a cow affected with reticulum cell sarcoma compared with a normal precrural lymph node.

FIG. 2. Nodular formations of reticulum cell sarcoma in the wall of the small intestine of a cow.

2



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Reticulum Cell Sarcoma of Lymph Nodes

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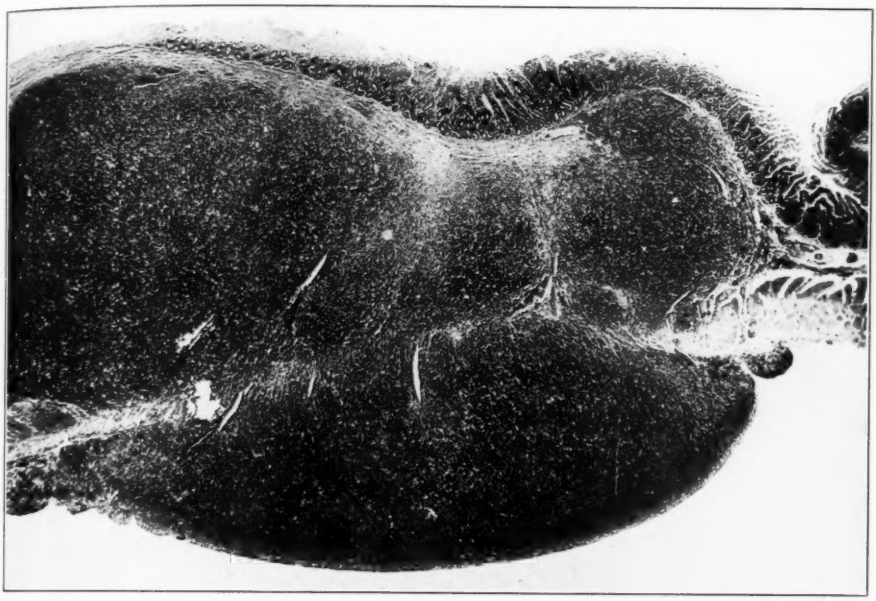
PLATE 74

FIG. 3. Section of the small intestine of a cow showing invasion and destruction of the entire wall by a reticulum cell sarcoma nodule. × 17.

FIG. 4. Section of heart muscle showing massive invasion and destruction of muscle fibers by reticulum cell sarcoma. × 22.



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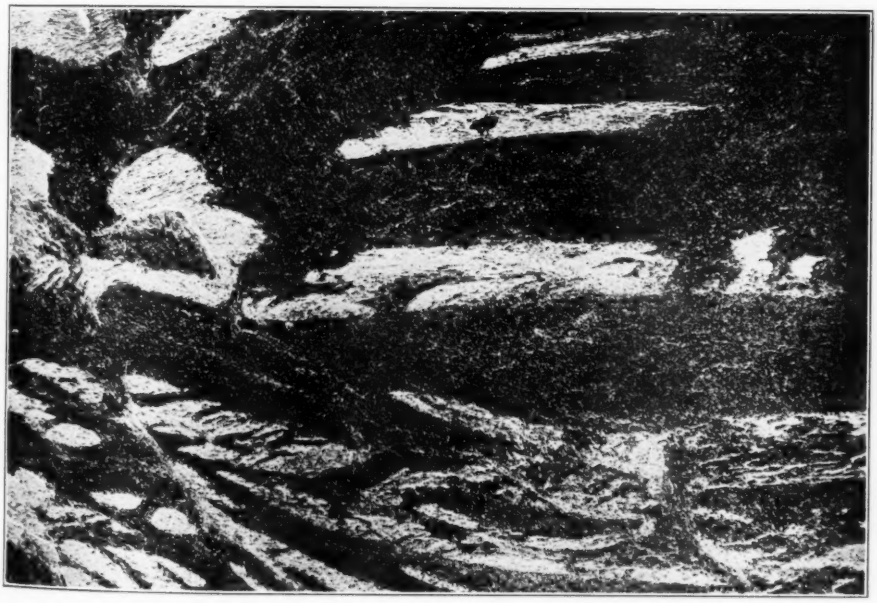

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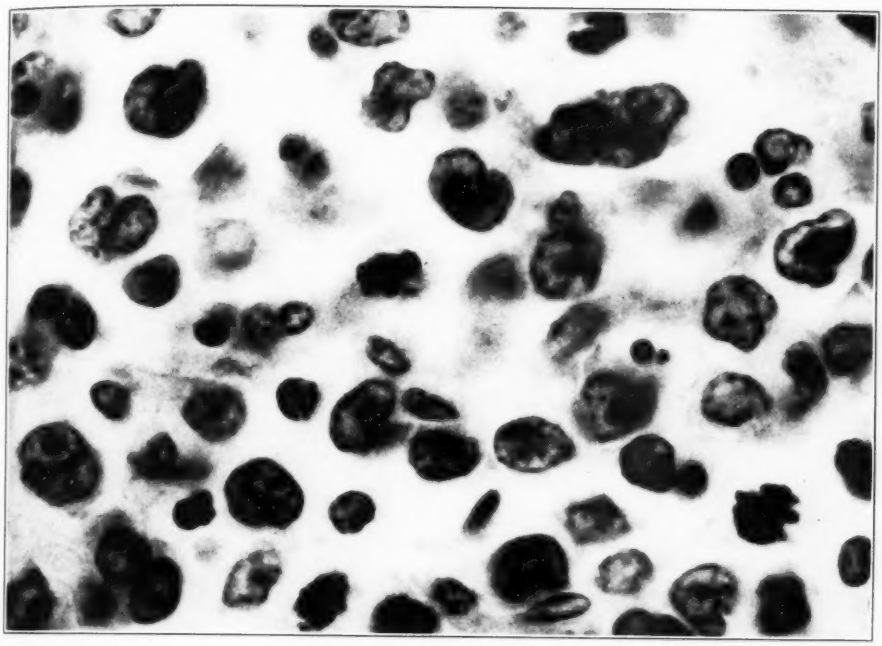
PLATE 75

FIG. 5. Section of lymph node affected with reticulum cell sarcoma. Note the irregularly shaped tumor cells and nuclei. Several mitotic figures are shown. × 1800.

FIG. 6. Same lymph node as shown in Fig. 5 impregnated with silver to show the network of delicate argyrophilic fibrils in direct contact with the tumor cells. × 775.



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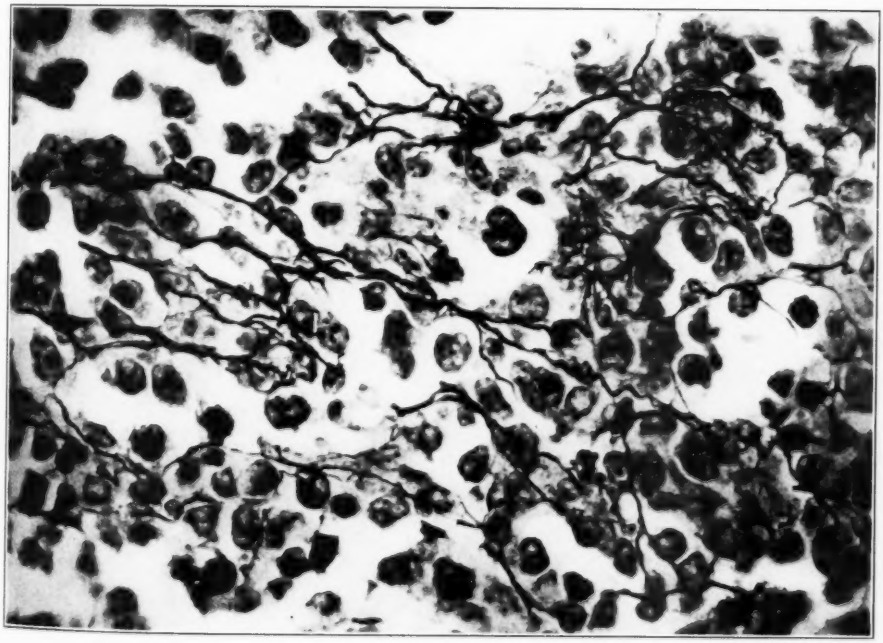

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EXPERIMENTAL INFECTION IN THE MOUSE PRODUCED BY INTRATRACHEAL INOCULATION WITH HEMOPHILUS PERTUSSIS *

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Numerous attempts to produce experimental infection in animals with *Hemophilus pertussis* have been made. In certain instances, particularly in the puppy,[1, 2] in the monkey,[3, 4] and in the chimpanzee,[5, 6, 7] the results have been encouraging. There can be little doubt that the disease has been experimentally established in the ape by Shibley [6] and by Rich and his co-workers.[7] MacDonald and MacDonald [8] have reported that typical pertussis resulted when the upper respiratory tracts of two children were inoculated with suspensions of *H. pertussis*. Gallavan and Goodpasture,[9] by inoculating the chorio-allantoic membrane or the amniotic fluid sac with *H. pertussis*, produced an experimental lung lesion in the chick embryo which they considered similar to that which occurs in the human disease.

During the past year we have been interested in the type of disease that results when the white mouse is inoculated intratracheally with recently isolated strains of *H. pertussis*. While the work was in progress Burnet and Timmins [10] reported results obtained by the intranasal inoculation of mice. It is interesting that our findings are similar to theirs, apparently the only difference being in the method of inoculation and in the dosage. Although our method is a bit more laborious, we apparently obtain fewer secondary invading organisms by lung cultures. Culotta, Marting and Liebow [11] by intranasal inoculation compared the type of lesion produced in the young with that produced in the more mature mouse and described the pulmonary lesion as being "characterized by an accumulation of cells along the course of the broncho-vascular rays." We have observed this lesion in our animals and have also added

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observations on the degree of leukocytosis that results from the infection.

Method

A standard suspension of *H. pertussis* is prepared by scraping a 72 hour growth from the surface of Bradford's medium [12] into 0.85 per cent NaCl solution. It is standardized to contain approximately 10 billion organisms per ml.

White mice, weighing from 15 to 20 gm., are anesthetized with ether in a glass jar. A small longitudinal incision is made over the ventral surface of the neck and the muscles retracted. By means of a bent probe, the trachea is brought into view and 0.05 ml. of the standard suspension of *H. pertussis* is introduced through a small needle from a tuberculin syringe. The cut edges of the skin are approximated and held in place by the application of a small amount of collodion. The entire procedure requires only a few minutes and less than 5 per cent of the mice are killed by it.

Results

With the dosage employed, from 60 to 75 per cent of the mice die after inoculation within from 1 to 10 days. The organism can be consistently recovered from the lungs, frequently in pure culture, for as long as 10 to 20 days after inoculation. During the first 3 days of infection, invasion of the blood stream occurs, as may be proved by making cultures of the peripheral blood from the tail, from the heart's blood, and from the spleen.

A definite respiratory wheeze, frequently persisting for several hours, results from the inoculation. During the next 24 hours the mouse becomes less active, refuses food, loses weight, and presents a roughened coat. Animals that die within 48 to 72 hours closely resemble those suffering from virulent pneumococcal infection.

When the mice are autopsied there is often a small amount of free bloody fluid in the serous cavities and small petechial hemorrhages in the peritoneum and pleura. The liver, spleen and kidneys are enlarged. The lungs are distended and patchy or diffuse hemorrhagic areas of consolidation are noted, particularly over the hilum and apical regions (Fig. 1). These areas are not depressed and are firm and rubbery. On sectioning the cut surface

is moist but, in places, granular. The bronchi contain frothy exudate.

Microscopically, stained sections of the lungs reveal typical interstitial changes with thickening of the alveolar walls and infiltration of mononuclear and polymorphonuclear leukocytes about the blood vessels and bronchioles (Fig. 2). During the first 72 hours after inoculation considerable amounts of mucus and cellular exudate accumulate within the alveolar spaces. Edema is pronounced and atelectatic areas are almost always present. After 3 weeks there is still evidence of the interstitial changes but the degree of polymorphonuclear leukocytic invasion of the parenchyma is much less marked (Fig. 3). Considerable mucus covers the epithelium of the bronchi in which many clumps of minute bacilli may be demonstrated by bacterial stains (Fig. 4). Some proliferation of the epithelium of the bronchus is noted, producing a distorted irregular contour of the epithelial layer of cells, but desquamation and fibrinoid necrosis apparently do not often occur. In this respect the lung lesion does not resemble that which is said to be characteristic of experimental infection with influenza virus.[13] Inclusion bodies have not been found.

Hyperleukocytosis

Counts made on blood samples obtained by clipping off a bit of the tail usually revealed a marked leukocytosis, frequently varying from 30,000 to 100,000 leukocytes per cubic millimeter (Table I). Normal counts by this method range between 10,000 and 20,000. The predominating type of cell is usually mononuclear, this type frequently representing 65 to 70 per cent of the total number of white cells. In the mouse the normal percentage of mononuclear cells in the blood ranges from 60 to 80 per cent.[14] In one mouse (A–3, Table I) the total leukocyte count reached 220,000 on the 5th day after inoculation. This animal died on the 10th day and culture of the lungs yielded a pure growth of *H. pertussis*. Table I illustrates the degree of leukocytosis produced by infection with *H. pertussis* as compared with that resulting from infection with an atypical pertussis-like organism [15] obtained from the upper respiratory tract of children suffering from pertussis. It is evident that the degree of leukocytosis is much greater in the mice infected with *H. pertussis*. Very little disturbance of the total

TABLE I

Comparison of Total Leukocyte Counts in Mice Intratracheally Infected with Hemophilus pertussis and with an Atypical Pertussis-like Organism Isolated from Cases of Suspected Pertussis

Mouse	Organism	Total leukocyte counts					Results	Lung cultures
		Before inoculation	After inoculation					
			2 days	5 days	10 days	15 days		
A–1	*H. pertussis*	17,500	66,000	124,000			D14*	+
A–2	"	21,600	27,500	50,000		30,000	K28*	o
A–3	"	12,800	30,000	220,000	180,000		D10	+ (pure)
A–4	"	16,000	25,000	39,000	84,500	67,500	K20	+
A–5	"	24,000	34,000	70,500			D10	+ (pure)
A–6	"	18,500	27,000	44,500	18,500	11,000	K20	o
A–7	"	15,000	8,500				D2	+
A–8	"	21,000	36,000				D2	+
A–9	"	14,000	11,000	44,500	42,500		D15	+ (pure)
B–1	Atypical pertussis-like organism	29,000	16,500	17,000			D15	o
B–2	"	18,000	38,000	62,000			D15	+
B–3	"	15,000	33,000	35,000		28,500	D22	o
B–4	"	16,500	23,500	6,500	26,500		K10	+
B–5	"	21,000	33,000	19,000	44,000		K15	+
B–6	"	22,500	45,000	45,000	19,500		K10	+
B–7	"	18,000	23,500	24,000	27,000		K15	o
B–8	"	14,500	19,500				K3	+
B–9	"	19,500	8,000				K5	+
C–1	Saline controls	13,500	24,500	16,500	24,000		K10	o
C–2	"	19,500	30,000	20,000	10,500		K10	o
C–3	"	20,000	28,000	24,000	22,500		K10	o

* D = died; K = killed; numerals represent the day after inoculation.

white cell count resulted when 0.85 per cent NaCl solution was introduced into the trachea.

Discussion

The type of lung infection in the mouse described in the present paper is apparently not specific for *H. pertussis*, because we have produced similar lesions with virulent strains of *H. influenzae* and with strains of atypical pertussis-like organisms that have some of the characteristics of *Brucella bronchisepticus*. In this respect, however, it may be recalled that Sprunt, Martin and Williams [16] showed that interstitial lesions produced in the lungs of rabbits by intratracheal inoculations of *Bacillus typhosus* closely resembled those produced by *H. pertussis*. Through the courtesy of Dr. Goodpasture it has been possible to compare the mouse lesion with the experimental lesion in the chick embryo and with that characteristic of human pertussis. As shown by Gallavan and Goodpasture,[9] the essential lesion in the chick embryo and in human pertussis is an invasion of polymorphonuclear leukocytes followed by necrosis of the basilar and midzonal area of the bronchial epithelium. Although we have observed some proliferation of the bronchial epithelium in the mouse, we have noted very little tendency of the polymorphonuclear leukocytes to invade this area. Necrosis of the epithelial cells has been observed but infrequently. On the other hand, we believe that the parenchymal lesion described above is more conspicuous and is the essential lesion produced by *H. pertussis* in the mouse lung.

The association of hyperleukocytosis with lung lesions in mice is interesting because the extremely high white cell count of the disease in the human is almost always associated with pneumonia. A comparison of the degree of leukocytosis produced by intratracheal with that produced by intraperitoneal inoculation of *H. pertussis* and a more detailed study of the degree of lymphocytosis is now being made.

That the mouse is susceptible to infection with *H. pertussis* is fortunate, because it affords an experimental infection that is readily available for study. Moreover, we have found this experimental disease useful in studying the virulence of different strains of *H. pertussis* and the protective qualities of various antigens and immune serums.

Summary

By the intratracheal inoculation of mice with suspensions of recently isolated *Hemophilus pertussis*, a characteristic lung lesion has been produced. It consists of an interstitial pneumonia and the development of an excess of mucoid exudate within the smaller bronchi and alveolar spaces. The parenchymal lesion is more conspicuous than that of the bronchial epithelium.

Pure cultures of *H. pertussis* have been consistently obtained from infected lungs from 10 to 20 days after inoculation.

The total leukocyte count has been regularly increased and extreme degrees of hyperleukocytosis have been observed occasionally.

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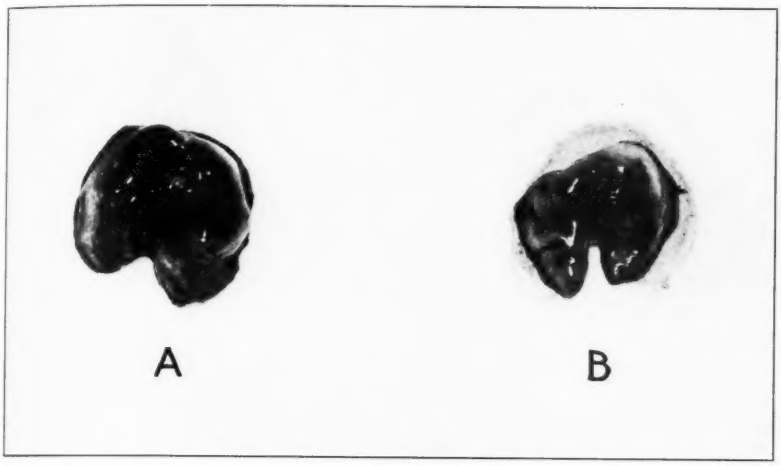
DESCRIPTION OF PLATES

PLATE 76

FIG. 1. A. Photograph of mouse lung showing typical 72 hour lesion produced by intratracheal inoculation of *H. pertussis*.
B. Photograph of normal mouse lung. × 2.

FIG. 2. Microphotograph of 72 hour lung lesion produced by intratracheal inoculation with *H. pertussis*, showing extensive alveolar and peribronchial cellular reaction and excessive exudate in the alveolar and bronchial lumens. × 210.



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Infection in Mouse with *Hemophilus pertussis*

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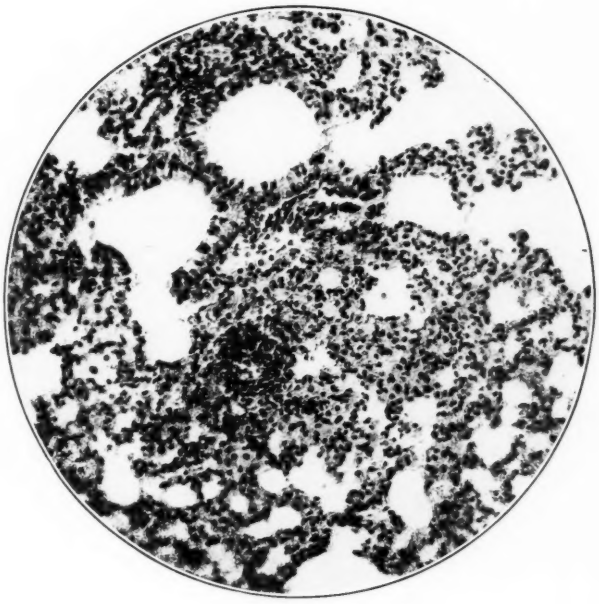
PLATE 77

FIG. 3. Microphotograph of 20 day lung lesion showing interstitial changes and cellular infiltration. × 210.

FIG. 4. Microphotograph of 20 day lung lesion. Bacterial stain showing clumps of *H. pertussis* in the bronchial epithelium. Pure culture of *H. pertussis* obtained from the lungs at autopsy. × 1500.



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